PROGRESS IN

Fundamental Medicine

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P I T D I NE C

PREFACE

This volume is made up of a series of authoritative discussions of topics of current clinical and pathologic importance The subjects have been chosen because information is being added about them The authors in each instance have contributed in a considerable degree to the understanding of the problem While the majority of these authors are grouped as pathologists each has an adequate appreciation of the clinical importance of tissue changes as the titles demonstrate This volume will reinforce the statement of the late W G MacCallum that the clinical sciences and pathology are simply different aspects of the one problem

For some time a volume of authoritative discussions has been needed The scientific journals multiply and portions of pertinent and important information are scattered here and there The abstract volumes do not fuse the data from the different articles into a satisfactory amalgam The newer material when it appears in a textbook is buried in the volume and is referred to in the preface as "Since the last edition the following new features have been added" Because of this situation important advances may fail to reach the attention of some doctors

The present volume should serve doctors in all of the different disciplines who are interested in obtaining significant recent contributions in a summary form The range of titles of the various articles indicates that the great majority of doctors will find individual subjects in which they are interested and much information which will be of value to them in their daily work That is the aim of pathology

The laboratory doctor will find recent methods reviewed and evaluated as well as pertinent concepts of disease important to them

J I A McMANUS M D

CHARLOTTESVILLE VIRGINIA

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Progress in Fundamental Medicine

Chapter 1

RECENT ADVANCES IN PARENTERAL NUTRITION WITH PARTICULAR REFERENCE TO PROTEIN HYDROLYSATES

By Paul R. Cannon, M.D.

Although the therapeutic usefulness of solutions of glucose, salts and vitamins as parenteral nutrients has long been recognized, a like recognition has not been accorded the protein hydrolysates. In fact this recent contribution to parenteral nutrition is still looked upon by many physicians and surgeons with caution and some scepticism. Furthermore many seem to believe that the loss of tissue nitrogen accompanying acute protein deficiency is of little metabolic concern in comparison with the loss of water, glycogen, salts or vitamins. It is obvious, nevertheless, that the metabolic debt resulting from the catabolism of tissue protein must eventually be repaid whether it be with reference to muscle, enzyme systems, blood plasma or hemoglobin, and that the penalty of nonpayment may be manifested during convalescence in terms of an extended period of asthenia and a delay in the restoration of tissues to their former structural and functional integrity.

Complacency with respect to the problems of tissue protein depletion seems all the more unfortunate when one takes into account the many pathologic consequences of a severe loss of important intracellular constituents incident to a prolonged period of negative nitrogen balance. Moreover, there is no longer so much justification for the overly cautious use of protein hydrolysates now that the hazards of antigenicity and pyrogenicity have been greatly reduced, the toxic reactions largely eliminated by the use of disposable tubing, and the cost materially lowered. This favorable outlook should not be taken to mean, however, that all obstacles in the field of parenteral nutrition have been triumphantly overcome; on the contrary this discussion will deal mainly with a number of problems still awaiting solution.

One of the oldest and most perplexing of these centers around the question of the nature of the so called "toxic destruction of protein," or the "catabolic phase" as it is now more usually called. In summarizing much of the evidence dealing with this subject Peters has emphasized the fact that in a variety of diseases the extravagant expenditure of tissue protein is so marked that despite large food intakes negative nitrogen balances cannot be prevented, and in patients with fractures Cuthbertson, Howard, Browne and others have demonstrated the extraordinary persistence of negative nitrogen balances for long periods despite high caloric and protein intakes. Grossman and associates have also reported instances of acute infection such as acute meningitis, or virus pneu

monia, in previously healthy subjects in which negative nitrogen balances were not perceptibly influenced by increases in the protein content of the diet It is apparent, therefore, that in healthy subjects acute infections and injuries may often lead to a loss of tissue nitrogen which seemingly cannot be counteracted by any known dietary means In contrast, malnourished subjects can utilize protein for reconstructive purposes surprisingly well It appears therefore that in many instances the maintenance of a positive nitrogen balance after trauma or infection depends to a large degree on the nutritional state of the subject However, until more is known as to the most desirable dietetic management of such patients Peters has advised a liberal protein intake since this entails no obvious deleterious effects and in consequence is preferable to protein limitation at the possible expense of delayed rehabilitation, and particularly in the debilitated subject there is good reason to feed a high protein and high calorie diet throughout the recovery period

Werner and his associates on the other hand have challenged the concept of catabolic injury and have pointed out that studies too often have failed to differentiate between the loss of nitrogen due to actual trauma and that due to a change in food intake in relation to the injury In short they suggested that the loss of nitrogen may be related more to an inadequate caloric intake than to the surgical procedure Fever according to them may also exaggerate the caloric needs and if the latter are not allowed for nitrogen loss will necessarily follow Thus infection even if at times inapparent may elevate caloric needs and account for some instances of so called catabolic loss of nitrogen Riegel and associates in emphasizing the extreme variations in nitrogen excretion in surgical patients with approximately similar nitrogen and caloric intakes have attributed these variations in part to such factors as anesthesia extent of operation degree of trauma previous nutritional history degree of postoperative pyrexia and other factors In any case they maintain that the intake of nitrogen customarily recommended for normal subjects will not maintain surgical patients after operation in a positive nitrogen balance However they were able to keep most of their patients in positive nitrogen balance during the early postoperative period (five days) by administering daily by mouth or by Miller Abbott tube at least 0 3 gram of nitrogen and 30 calories per kilogram of body weight

In an experimental study of this problem Madden has suggested that the underlying mechanism of the catabolic phase is an accelerated breakdown of protein rather than an interference with anabolism For example when he injected radioactive methionine intravenously together with an adequate mixture of all the other essential amino acids he observed in normal animals no increased output of nitrogen or sulphur If however the same mixture was injected into animals which had been injured by the production of turpentine abscesses there was a marked excretion of both nitrogen and sulphur although tissue analysis failed to reveal any difference in radioactivity in the two groups of animals This evidently means that methionine was built up into tissue protein even after trauma from the abscess and that although utilization of the amino acids was hampered somewhat by the abscess some benefits were obtained from their use It is probable therefore that even though it may be difficult and at times impossible to prevent a considerable loss of body nitrogen following trauma further elucidation of the general nature of protein metabolism may help to improve the processes of rehabilitation despite the fact that preponderant evi

dence points to a severe alteration of the normal processes of protein metabolism Thus Man *et al* have reported an abrupt fall in the concentration of amino acids in the plasma and a tendency for them to remain at low levels until recovery is well along The extent of the fall seems to be directly proportional to the severity of the operative procedure These findings are in agreement with the views of others (Cuthbertson, Howard Browne) that the severity of the catabolic process is related to the intensity of the causative factor and the response of the host

It has been suggested that the adrenal cortex and the pituitary gland may exert control over these catabolic processes but experimental evidence at least with respect to the adrenal does not seem to support this point of view For example Ingle and associates in a study of fractures in adult rats reported that "the negative nitrogen balance which characteristically develops following fracture may require the presence of the adrenal cortical hormones but is not caused specifically by the increase in secretion of cortical hormones which occurs during stress Noble and Toby, in a somewhat similar study came to similar conclusions viz that catabolism after trauma occurs independently of the adrenal cortex"

Aside from the question of increased caloric need there is a possibility that a more intensified use of essential amino acids, salts or vitamins may help further to reduce the catabolic loss of nitrogen For example, Emerson and Binkley, in treating patients with penetrating chest wounds found that dietary supplementation by intravenous feeding of a mixture of ten essential amino acids increased the degree of utilization of nitrogen to as much as 2 or 3 times the total increase in nitrogen intake They suggested in explanation that regeneration of damaged tissue may take precedence over and at the expense of metabolism of normal tissue thus requiring additional essential amino acids if "raiding of other tissues is to be prevented This point of view coincides essentially with that of Croft and Peters with respect to the value of methionine supplementation in the treatment of burns It is possible therefore, that a more intensive use of protein hydrolysates may have a similarly beneficial effect

Granted the possibility of preventing or minimizing the postoperative or post traumatic loss of tissue nitrogen what additional evidence may be cited indicating a need for a more general use of parenteral alimentation? In the first place the assumption seems warranted that in general neither a lack of appetite nor an inability to retain food justifies the conclusion that an adequate food intake is not desirable On the contrary accumulating evidence indicates that the processes of protein metabolism are so dynamic that even under optimal nutritional conditions the daily loss of protein in an average adult approximates 25 grams a loss which must be replaced each day if nitrogen equilibrium is to be maintained This daily requirement for dietary protein operates continuously and represents the inevitable and steady erosion of the bodily machinery and inasmuch as this nitrogen comes from cellular protoplasm its daily replacement would seem to be a metabolic necessity Furthermore because many poor risk surgical patients are unable to retain food by mouth or to eat it in adequate amounts realimentation should improve their nutritional status and thus prepare them for the ordeal of major surgery In operations on the colon or stomach it is advisable to have the gastrointestinal tract as empty as possible and Duncan Mirick and Howard have shown that after intravenous alimentation the in

testinal tract is empty and in good condition for surgical manipulation Post operatively, also the tendency to gaseous distention, nausea and vomiting carries with it the hazards of aspirative pneumonia, wound disruption ulcerative esophagitis etc Moreover if good nutrition is maintained both before and after operation there is a better chance for early ambulation, less postoperative asthenia, less tendency to urinary retention and better wound healing It is because of the considerable amount of vomiting diarrhea or distention which may occur in the early postoperative period that Riegel *et al* suggested the particular nutritive value of intravenous feeding in the first forty eight to seventy two hours after operation

In this discussion an effort will be made to adhere so far as possible to the principle that nutriment should be given parenterally only when it cannot or should not be given by mouth, whether because it cannot be ingested in adequate amounts or because of contraindications to its use in that way There are numerous situations in which these principles apply *viz* in obstructive lesions of the gastrointestinal tract, such as cancers intestinal obstructions intestinal fistulas, gun shot wounds of the abdomen ulcerative colitis, regional enteritis, perforated peptic ulcer, peritonitis and following gastrectomy or esophagectomy, as well as in such diverse conditions as coma meningitis, brain tumor, skull fracture, dementia unconsciousness etc In any case parenteral alimentation should be looked upon as a temporary expedient to be resorted to only until the patient is again able to take food by mouth in adequate amounts There may be particular urgency now also in the possibility that military exigencies may soon require parenteral alimentation in patients who are unable to take nourishment by mouth and for whom adequate amounts of blood or plasma may not be available at least for prolonged use

It is rather widely assumed at the present time that if blood plasma is readily available there is little reason to be concerned about the use of protein hydrolysates This assumption is due in part to the emphasis upon the importance of plasma protein concentration This emphasis however has also tended to obscure the fact that hypoproteinemia is an *effect* of protein deficiency and not a *cause* In consequence the intravenous injection of plasma may give a false sense of security if one regards plasma protein concentration as a primary problem For example Weech Goettsch and Reeves reported that less than 5 per cent of the nitrogen lost by dogs on a low protein diet could be accounted for by the decline in total circulating serum albumin Sachar and associates also observed in dogs that in protein depletion approximately 30 grams of body protein were lost for each gram of plasma protein and that for repletion about 30 grams of protein were required for the body tissues for every gram of plasma protein Obviously plasma protein concentration cannot fail to increase whenever colloids such as plasma or serum albumin are injected into the blood stream in amounts large enough to cause them to accumulate at a faster rate than they can be degraded and utilized The basic problem remains however namely the general tissue protein deficit and the correction of this deficit in order to reestablish effective functioning of the cells which synthesize enzymes plasma protein hemoglobin etc Admitting the unquestioned therapeutic value of plasma protein as an osmotic agent promoting restoration of blood volume its use as a means of prolonged nutriment presents disadvantages which must be carefully evaluated

A fact too often underemphasized is that plasma is an expensive commodity which is economically unavailable to most persons except in times of great catastrophe and its associated large scale blood donations To rely upon free blood plasma in ordinary times is to raise the related question Why should plasma be free while other medical items must be paid for? But if plasma is purchased a liter will usually require 4 donors for each 2 liters of blood (Elman) At customary rates this may cost $70 or more For example, a recent quotation on lyophilized and irradiated plasma supplied by the American Hospital Supply Corporation listed the material at $36 per 500 cc If the demand should become excessive it is doubtful moreover that it could continue to be supplied at this rate, if at all A liter of such plasma contains approximately 60 to 70 grams of protein Thus the cost of its protein would amount to more than one dollar per gram and this amount of protein is the equivalent of about two thirds of a pound of lean meat costing not more than one cent a gram for the protein in it Under such circumstances plasma protein would have to be a superior protein indeed to justify paying a hundred times or more the cost of meat protein, provided of course that the nutritive equivalent of the latter could be made available in other ways Furthermore the problem of collection of large quantities of plasma for use in times of disaster represents an expenditure of time and money which would be justified only if this is the one and only solution to the problem of intravenous nutrition and if the hazards of homologous serum jaundice plasma anemia toxic reactions costs of blood banks etc are also ignored But in view of the not infrequently fatal consequences of homologous serum jaundice it would seem unwise to take chances unless absolutely necessary Thus Lipman, in commenting recently on a case of fatal virus hepatitis in an infant three months of age remarked that it would be wise to be circumspect regarding the use of plasma in infants and children and for general supportive therapy to utilize various protein hydrolysates instead of plasma ' Finally unless plasma can be given simultaneously with an adequate caloric intake there is no reason to suppose that its protein will be any more effectively utilized than is the case for dietary protein in any other subcaloric state and to use plasma protein as a source of calories under such circumstances is to compound unjustifiably the expense of this protein as a nutrient

Aside from these practical considerations what is the evidence that plasma is a desirable protein for intravenous nutrition? Here we are at least on firmer ground and certainly there can be no doubt about the fact that plasma protein is a high quality protein which can be utilized for tissue protein synthesis It is indeed the main constituent of the circulating protein pool whose significance has been so well revealed by Whipple and his associates Thus in 1934 Holman Mahoney and Whipple showed that dogs fed only sugar by mouth could be maintained in nitrogen balance by the intravenous injection of dog plasma In the following year Pommerenke *et al* confirmed this work and demonstrated that the parenteral injection of dog plasma into a protein fasting dog enabled the animal to stay in nitrogen equilibrium with no surplus elimination of nitrogen after the experiments were ended They too concluded that plasma protein can be utilized efficiently either to replace or to repair tissue protein

These early findings have now been abundantly confirmed both in lower animals and in man and Eckhardt and Davidson have demonstrated in a human patient that an acid hydrolysate of human serum albumin reinforced with 1

tryptophan, 'contained all the essential amino acids required by man for maintenance of nitrogen balance and weight and for tissue protein repletion when administered in adequate amounts by vein for short periods of time.' Fletcher and associates also gave serum albumin reinforced with dl-acetyltryptophan intravenously to 11 surgical patients as the sole source of nitrogen and found that 3 remained in nitrogen equilibrium and 8 in positive nitrogen balance when the albumin was administered under conditions of 0.2 grams of nitrogen per kilogram of body weight and 25 calories per kilogram per day for five postoperative days in patients who had undergone major abdominal surgery.

There is evidence, however, that the utilization of homologous plasma is relatively slow and that the degradation rate of injected plasma to an assimilable form may proceed at a rate perhaps not faster than 10 per cent per day (Yuile *et al.*). Terry and associates have shown, also, that although they could keep dogs in health and nitrogen balance for as long as three months with homologous plasma as the only source of dietary nitrogen, the repeated daily injection of such plasma led eventually to a marked hyperproteinemia and, at a certain stage, to proteinuria. Evidently the injection of plasma at rates faster than it was utilized led to its retention and accumulation in the circulating blood at mounting rates of concentration. Eckhardt and associates have observed a similar effect following the intravenous injection of serum albumin into healthy young men. They concluded that the injected albumin rapidly diffuses from the plasma into the lymphatic system and establishes an equilibrium between the vascular and extravascular albumin. The albumin retained in the body undergoes a process of degradation or decay in the course of which amino acids are released for cellular use. They argue that it is because of this slow rate of degradation that albumin is an ideal substance for temporarily increasing the plasma albumin concentration and the oncotic pressure of the blood. The 50 per cent disappearance time was estimated by them to be between four and six days. Albright and associates have also reported that following the intravenous administration of plasma protein into a normal individual, burning and conversion of the protein did not start to an appreciable degree until at least the third post-injection day and that approximately 50 per cent of the injected plasma protein was eventually burned and the remainder converted into tissue protein.

Several studies in human subjects have also revealed hazards in the use of homologous serum albumin due to its effect upon blood volume and the slow rate of utilization. For example, Gimbel, Riegel and Glenn found that the injection into normal human subjects of human serum albumin supplemented with dl-acetyltryptophan led to its accumulation in the plasma, interstitial fluid and lymph, associated later with albuminuria, and that congestive heart failure developed in healthy young males receiving as little as 50 grams of albumin intravenously for one week. They concluded that 'the phenomena of circulatory overloading seriously hamper the usefulness of albumin as a nutritional supplement beyond its well established value in making up actual deficits of serum albumin.' It should be reemphasized, however, that protein-depleted persons apparently do not react in this fashion so long as they exhibit the tissue protein deficits. Waterhouse and associates have also studied the utilization of purified human serum albumin in human subjects with essentially similar results. Thus in a patient convalescing from rheumatic disease they observed the development of an intense proteinuria without evidence of impairment of renal function

in a woman with probable generalized vascular disease there developed peripheral edema, hydrothorax and pericardial effusion with poor utilization of the injected protein, and in a young woman in fair health signs of cardiorespiratory difficulty appeared They suggested, therefore that the utilization of such material depends largely on its osmotic properties and its slow rate of degradation so that when it is given in large quantities it accumulates and exerts its osmotic effects In giving it, therefore one must bear in mind the state of the capillaries in general and that of the renal glomeruli in particular as well as the status of the cardiac reserves The proteinuria is merely the expression of the exceeding of the maximal absorptive capacities of the renal tubules rather than an evidence of renal injury

The enumeration of these difficulties related to the use of human plasma or serum albumin merely brings into the foreground the metabolic problems which may possibly be better solved by the use of other kinds of nitrogenous nutriment Perhaps the ultimate solution of the problem will be found in the early and cautious use of blood, plasma or albumin for their immediate effects upon blood volume followed by the use of less expensive protein hydrolysates as the source of amino acids for tissue needs until food can again be eaten

CALORIC DEFICIENCY IN RELATION TO PROTEIN SYNTHESIS

The most serious obstacle to the more general employment of intravenous alimentation is caloric deficiency, and unfortunately there is no practical way now known of supplying enough calories by the intravenous route to ensure optimal utilization of injected amino acids for the purposes of tissue protein synthesis Nevertheless the fact cannot be ignored that optimal synthesis can occur only in the presence of an adequate caloric intake For example, in experiments designed to ascertain the influence of caloric intake upon tissue protein synthesis in protein depleted rats (Benditt *et al*) groups of animals were fed diets in which the total daily ingestion of protein was equal but the caloric intake differed Under such circumstances the capacity of the animals to form new tissue protein varied directly with caloric intake and at the lower levels of intake the utilization of protein was minimal Under similar circumstances in a patient it is not likely that intravenously injected nitrogenous material can be utilized optimally unless there is an adequate intake of calories and the utilization of the nitrogenous nutriment as fuel will obviously divert it from its primary purpose, namely, to supply tissue building material

It has been suggested that the need for exogenous calories may not be so urgent as is commonly assumed because of the utilization of body fat as a source of reserve calories Rice has suggested however that we have been guilty of soothing our minds in this respect In view of the lack of evidence substantiating the ready utilizability of such a source of calories it would seem to be unwise to rely upon this source of energy at least until more proof is at hand that body fat can be utilized at rates commensurate with the dynamic requirements of protein metabolism Indeed Pareira Probstein and Somogyi even argue against the employment of parenteral hydrolysate therapy in subcaloric states both because the body is forced to use the amino acids as fuel with little opportunity to convert them into plasma or tissue proteins and because the liberation of

tryptophan, 'contained all the essential amino acids required by man for maintenance of nitrogen balance and weight and for tissue protein repletion, when administered in adequate amounts by vein for short periods of time' Fletcher and associates also gave serum albumin reinforced with dl acetyltryptophan intravenously to 11 surgical patients as the sole source of nitrogen and found that 3 remained in nitrogen equilibrium and 8 in positive nitrogen balance when the albumin was administered under conditions of 0 2 grams of nitrogen per kilogram of body weight and 25 calories per kilogram per day, for five postoperative days in patients who had undergone major abdominal surgery

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point of view protein hydrolysates with glucose would be given because of the presumption that it is sound physiologic practice to maintain the tissue nitrogen stores at as near the equilibrium level as possible

In a further effort to increase caloric intake the addition of alcohol to hydrolysates has been practiced with some success For example, Rice and associates have used a mixture of dextrose alcohol and amino acids in the parenteral feeding of several hundred surgical patients They usually gave about 2 liters of solution in the first twelve hours after operation, thereby providing the patient with 100 grams of amino acids, 100 grams of dextrose and 120 cc of alcohol with a total caloric potentiality of almost 1500 calories Food was offered the following morning and, if accepted, no further parenteral therapy was given Otherwise both oral and parenteral feedings were given until the patient could eat well The alcohol has the added advantage of providing some sedation, thus cutting down on the need for morphine The authors believe that under these conditions it is easier to achieve positive nitrogen balances and that the calories from alcohol spare nitrogen Water soluble vitamins and electrolytes were also given parenterally when indicated Grabill *et al* have also reported similar favorable effects with the use of alcohol parenterally

Because of the limited utilizability of glucose solution when injected intravenously, attention is now being directed to the possibility of using solutions of invert sugar in which half of the carbohydrate is fructose For example, Weinstein has reported that the assimilation of invert solution is superior to that of glucose alone with a consequently diminished loss of calories It is claimed indeed that a 10 per cent solution of invert sugar can be injected intravenously as rapidly as can a 5 per cent solution of glucose and with almost complete utilization Evidence has been presented also by Weichselbaum and associates that fructose is a better glycogen former than glucose and that in other ways it may be an effective carbohydrate for metabolic needs At any rate further evidence must be awaited before it will be possible to ascertain to what extent progress has been made toward the goal of caloric adequacy by the intravenous route

Undoubtedly the greatest need, however is for a fat emulsion suitable for intravenous use and capable of supplying at least 2000 calories per day Gratifying progress has indeed been made in the development of such an emulsion and several types have been given clinical trial with no apparent ill effects (Holt *et al* Clark and Brunschwig) Unfortunately however it has not been possible as yet to prepare a fat emulsion which does not become toxic upon standing for a few weeks and until this difficulty can be overcome the extensive use of fat emulsions will be necessarily delayed

In a series of papers Stare and his associates have described their efforts to prepare stabilized fat emulsions for intravenous use After overcoming earlier difficulties associated with the tendency of the emulsions to cause granulomatous lesions and vasopressor reactions they finally succeeded in preparing a type of emulsion which when given intravenously in human subjects caused no unfavorable reactions and evidenced at autopsy no gross or microscopical evidence of pathologic changes related to the fat infusions These emulsions exerted no osmotic effects and had a favorable influence upon nitrogen and potassium balances and the prevention of weight loss Unfortunately as mentioned above they tended to become pyrogenic in about four weeks and had to be freshly prepared for clinical use It is to be hoped that further efforts to emulsify and

ketone bodies during deamination imposes an undue burden on the metabolic mechanisms Therefore, until it becomes practicable to supply an adequate intake of calories parenterally in the postoperative state these workers question the advisability of the attempt to give amino acid solutions at all In their opinion 'the furnishing of an adequate calorie supply parenterally in the post operative state is difficult and scarcely practicable But even if it were feasible it has not yet been definitely established whether or not nitrogen can be efficiently utilized in the post traumatic state under any circumstances'

In an effort to overcome the calorie difficulty the usual procedure is to inject solutions of glucose most commonly in 5 per cent concentrations Under such circumstances, however it is practically impossible to supply even the daily caloric needs for maintenance For example 3 liters of 5 per cent glucose solution will furnish less than 600 calories per day whereas the bodily needs in a 60 kilo gram subject are nearer 2000 to 2400 non protein calories, and intakes well below this will undoubtedly be inadequate for optimal utilization of amino acids, either for tissue protein reconstruction or for maintenance Thus Ravdin and Gimbel have reported that at least 30 calories per kilogram of body weight' were essential to achieve nitrogen balances, no matter how much protein was given, and in the experiments of W C Rose where amino acid mixtures were fed as the sole source of dietary nitrogen it was necessary in some instances to give as high as 55 calories per kilogram of body weight The reasons for this unusual caloric need are still obscure but the fact cannot be ignored

In an effort to increase the caloric intake, concentrations of glucose as high as 25 per cent have been employed Unfortunately, the likelihood of injury to veins is also correspondingly increased thus militating against the prolonged use of such solutions Ellison and his associates have presented convincing evidence however *that the efficiency of parenteral alimentation can be materially improved* by the use of 10 per cent and 15 per cent solutions of glucose in association with 5 per cent amigen In protein depleted human subjects they demonstrated a direct relation between protein utilization and the total caloric intake when both nutrients were administered simultaneously The choice between 10 per cent and 15 per cent solutions of glucose depends in their opinion upon the therapeutic goal If only maintenance is aimed at 3 liters per day of 10 per cent solution of glucose in 5 per cent amigen was adequate whereas in order to ac complish restitution of tissue protein and weight gain it is advisable to give 3 liters per day of 15 per cent solution of glucose in 5 per cent amigen

It has been commonly assumed that glucose solutions when injected intra venously can be utilized at a rate of approximately 0 85 grams per kilogram per hour (Woodyatt *et al*) Newer evidence indicates however that this figure is in many instances too high and that only about 0 5 gram per kilogram per hour can be metabolized Amounts above this rate are therefore likely to be lost in the urine (Cooper *et al* Lockhardt and Elman) If the latter rates are taken into account the utilization of 150 grams of glucose in 3 liters of 5 per cent glucose in a 50 kilogram subject would require six hours of infusion time to give 600 calories and about twelve hours if 300 grams were given in 10 per cent solution It is obvious *therefore that* until this problem of caloric intake can be solved the most that can be hoped for is a compromise sort of 'holding operation in which the main effort is to minimize the loss of tissue nitrogen rather than to accomplish any marked degree of tissue protein repletion According to this

point of view protein hydrolysates with glucose would be given because of the presumption that it is sound physiologic practice to maintain the tissue nitrogen stores at as near the equilibrium level as possible

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ketone bodies during deamination imposes an undue burden on the metabolic mechanisms Therefore until it becomes practicable to supply an adequate intake of calories parenterally in the postoperative state these workers question the advisability of the attempt to give amino acid solutions at all In their opinion "the furnishing of an adequate calorie supply parenterally in the post operative state is difficult and scarcely practicable But even if it were feasible, it has not yet been definitely established whether or not nitrogen can be efficiently utilized in the post traumatic state under any circumstances"

In an effort to overcome the calorie difficulty the usual procedure is to inject solutions of glucose, most commonly in 5 per cent concentrations Under such circumstances, however, it is practically impossible to supply even the daily caloric needs for maintenance For example 3 liters of 5 per cent glucose solution will furnish less than 600 calories per day whereas the bodily needs in a 60 kilogram subject are nearer 2000 to 2400 non protein calories and intakes well below this will undoubtedly be inadequate for optimal utilization of amino acids either for tissue protein reconstruction or for maintenance Thus, Ravdin and Gimbel have reported that at least 30 calories per kilogram of body weight" were essential to achieve nitrogen balances, no matter how much protein was given, and in the experiments of W C Rose, where amino acid mixtures were fed as the sole source of dietary nitrogen, it was necessary in some instances to give as high as 55 calories per kilogram of body weight The reasons for this unusual caloric need are still obscure but the fact cannot be ignored

In an effort to increase the caloric intake concentrations of glucose as high as 25 per cent have been employed Unfortunately, the likelihood of injury to veins is also correspondingly increased, thus militating against the prolonged use of such solutions Ellison and his associates have presented convincing evidence however that the efficiency of parenteral alimentation can be materially improved by the use of 10 per cent and 15 per cent solutions of glucose in association with 5 per cent amigen In protein depleted human subjects they demonstrated a *direct relation between protein utilization and the total caloric intake when* both nutrients were administered simultaneously The choice between 10 per cent and 15 per cent solutions of glucose depends in their opinion upon the therapeutic goal If only maintenance is aimed at 3 liters per day of 10 per cent solution of glucose in 5 per cent amigen was adequate whereas in order to accomplish restitution of tissue protein and weight gain it is advisable to give 3 liters per day of 15 per cent solution of glucose in 5 per cent amigen

It has been commonly assumed that glucose solutions when injected intravenously can be utilized at a rate of approximately 0 85 grams per kilogram per hour (*Woodyatt et al*) *Newer evidence indicates however* that this figure is in many instances too high and that only about 0 5 gram per kilogram per hour can be metabolized Amounts above this rate are therefore likely to be lost in the urine (Cooper *et al* Lockhardt and Elman) If the latter rates are taken into account the utilization of 150 grams of glucose in 3 liters of 5 per cent glucose in a 50 kilogram subject would require six hours of infusion time to give 600 calories and about twelve hours if 300 grams were given in 10 per cent solution It is obvious therefore, that until this problem of caloric intake can be solved the most that can be hoped for is a compromise sort of holding operation in which the main effort is to minimize the loss of tissue nitrogen rather than to accomplish any marked degree of tissue protein repletion According to this

restoration of the depleted potassium stores, a deficiency of potassium might be expected to act as a limiting factor and thus retard the processes of tissue protein repletion, particularly of muscle Experimental evidence suggests that this is so, in that a protein depleted rat subjected to protein repletion even though supplied all dietary essentials except potassium loses appetite and fails to regain lost weight effectively until potassium is added to the repletion diet (Cannon Frazier and Hughes)

Probably all of the protein hydrolysates now being used for parenteral alimentation are low in potassium content Moreover, many patients receiving intravenous hydrolysate therapy have also had large amounts of saline and glucose solution administered or have other conditions which lead to a loss of potassium, as for example severe diarrhea vomiting marked weight loss hemorrhage shock burns acidosis alkalosis, etc It is likely therefore that a focusing of attention upon the potassium needs will bring about further improvement leading to a more effective utilization of the protein hydrolysates Attention should be paid also, to the needs for phosphorus and magnesium in view of the fact that these salts together with potassium, are the three principal electrolytes concerned with intracellular metabolism There is little doubt now that the continued use of such overly simplified electrolyte solutions as salt and glucose solution will contribute little to the more efficient use of the protein hydrolysates and that the needs for intracellular electrolytes must be given more consideration

Not much is known as yet concerning the exact role of potassium or of phosphorus in cell metabolism other than that both elements play an essential part in phosphorylating reactions and in the building up of so called high-energy phosphate bonds The role of potassium in protein synthesis is also unknown although it is known that it is necessary for muscle contraction It is not surprising, therefore that in such an active muscular organ as the heart a potassium deficiency should adversely affect the muscle fibers At any rate potassium presumably has multiple functions and some of them are probably independent of nitrogen deposition Thus Howard has reported that in the catabolic phase of protein metabolism after fracture, and with a good dietary intake there may be a positive potassium balance during a period of heavy loss of nitrogen

There is increasing evidence however of the relationship of potassium deficiency to muscular weakness This has been demonstrated particularly in familial periodic paralysis but it has also been observed in patients receiving large quantities of saline and glucose solution in the treatment of acidosis alkalosis diabetic coma etc Probably the most serious consequence is that of myocardial weakness and heart failure and there is experimental evidence (Cannon Frazier and Hughes) that extensive lesions appear in the hearts of protein depleted rats subjected to severe potassium deficiency Moreover these animals often die in congestive heart failure However the lesions quickly disappear when potassium is administered indicating that the loss of myocardial intracellular substance can be prevented by an adequate intake of potassium

It is of interest to consider the metabolic situation when human plasma is used as a sole source of intravenous protein nutriment in a debilitated patient who has lost both tissue nitrogen and potassium If one assumes that for nitrogen balance with an otherwise adequate diet the nitrogen turnover is in the neighborhood of 4 grams per day and that under such circumstances approximately 400 milligrams of potassium may be excreted as well the inference might be drawn

stabilize fat emulsions by other methods may in time eliminate some of the current preparational difficulties

Shafiroff and associates have also prepared fat emulsions in which homogenization was accomplished by the use of gelatin In this way they obviated the use of phosphatides which, in their opinion, are most probably responsible for the toxic reactions After injecting the emulsion into dogs without the development of toxic reactions, secondary anemia or other abnormality, they prepared fat emulsions containing amigen and glucose with a caloric value of 1300 calories per liter These they gave with safety to 76 human subjects Later they increased the caloric potentiality to as high as 2100 calories per liter by using a fat concentration of 20 per cent The emulsions remained stable with respect to particle size within the 1 micron range for at least seven months These emulsions were administered to 25 human subjects in the course of 51 infusions Although increased temperatures or chills developed in about 27 per cent of the infusions given, the authors thought that about half of these were due to the too rapid administration of the preparations

A fat emulsion has also been described by Meng and Early which has been infused into healthy dogs for as long as four weeks with the animals remaining healthy and in good spirits Although this preparation has not yet been used in human subjects the authors believe it 'could be used clinically with considerable confidence, and that it would be very beneficial in cases where complete parenteral alimentation is necessary

THE ROLE OF INTRACELLULAR ELECTROLYTES IN AMINO ACID UTILIZATION

Because of the loss of tissue protein as a consequence of debilitating disease it is not surprising that there should be a loss also of intracellular electrolytes For example in the course of muscle atrophy there is both a loss of tissue nitrogen and of other intracellular constituents of muscle as well including potassium phosphate magnesium vitamins and enzymes To the extent therefore that the regeneration of atrophic muscle requires the integrated action and simultaneous utilization of various dietary essentials which enter into the composition of this tissue it is probable that a lack of any one of these might seriously interfere with tissue reconstruction Indeed evidence now accumulating points increasingly to the vital importance of potassium and phosphate in the utilization of amino acids for tissue protein synthesis Although potassium has long been known to be essential for growth of plants and animals recent evidence also indicates its essentiality *in the processes of tissue protein reconstruction* Several workers have demonstrated that during fasting the urinary loss of nitrogen and potassium approximates a ratio of 10 1 this ratio also obtains in normal muscle (Fenn) The inference is that much of the loss of nitrogen and potassium is a consequence of muscle wastage

Of the approximately 150 grams of potassium present in the body of a 70 kilogram man about 115 grams are present in muscle (Donegan) Thus such an individual losing about 20 per cent of his muscle mass would also lose about 23 grams of potassium When an attempt is made therefore to rebuild his depleted tissue protein stores by parenteral feeding but without provision for the

value However not all are of equal nutritive value, and there is a need for a method or methods of assay which can reveal the hydrolysates which are nutritively superior The methods customarily used have consisted of amino acid analyses both chemical and microbiological nitrogen balance determinations in animals and man, growth tests and protein repletion tests The hydrolysates have been evaluated either by means of ingestion or parenteral administration Due to a lack of uniformity of methods, however no generally agreed upon procedure has been adopted No doubt in time a method will be developed which will at least ensure therapeutic acceptance of those hydrolysates which are safe and reasonably nutritious, and exclude those of negligible nutritional value Fortunately at the present time there is no evidence that any now available fall in the latter category In time, moreover, it will probably be possible to demonstrate which preparations are most suitable for parenteral use in human subjects When that is accomplished it will be possible to improve further the methods of preparation and of use Until then however much needs to be learned about the nutritive potentialities of the hydrolysates now being sold for therapeutic use

THE PROBLEM OF THE EVALUATION OF THE PROTEIN HYDROLYSATES

In the nutritive evaluation of the hydrolysates now in clinical use or to be developed the following criteria are essential

1 The preparations must be non toxic (non antigenic and non pyrogenic)
2 They must contain all the amino acids indispensable for man, and in proper amounts and proportions
3 They must contain adequate amounts of non essential amino acid nitrogen in order to 'spare' essential amino acids
4 The amino acids must be in a form available for tissue utilization when given parenterally

As has been already pointed out with reference to the first point improvements in preparation and the use of disposable tubing have eliminated most of the hazards of toxic reactions At present therefore hydrolysates now available have been widely used with a minimum of undesirable reactions The problem of nausea and vomiting still remains but further improvements in the preparation and modes of administration will probably eliminate these difficulties in time

In considering the question of the content of essential amino acids in a particular hydrolysate there can no longer be any question concerning the importance of the 'limiting action of essential amino acids, nor that in man eight are required in definite daily amounts (Rose) and proportions for the maintenance of nitrogen equilibrium There is reason to believe also, (Steffee) that for the repletion of protein depleted tissues there is an augmented need for essential amino acids which should be allowed for in any situation requiring nutritional rehabilitation

In the evaluation of a protein hydrolysate the first requirement is to demonstrate the presence in it of all the essential amino acids This is a simple procedure because if any one essential amino acid is present in a low amount the fact can be readily demonstrated by a rat growth or rat repletion method If the animals show a reluctance to eat the material the most probable cause is an essential

that to maintain nitrogen equilibrium even at this low maintenance level of nitrogen turnover at least 400 milligrams of potassium might be needed daily In view of the fact however, that a liter of plasma contains only about 200 milligrams of potassium it is evident that 2 liters of plasma daily would barely supply the replacement needs for potassium which had been lost And for the reconstruction of depleted tissues more nitrogen and potassium would undoubtedly be needed Therefore, even when plasma is used as a parenteral nutriment there is reason to believe that supplementation with potassium would be advantageous and possibly essential

TYPES OF PROTEIN HYDROLYSATES

In the development of protein hydrolysates for parenteral use the protein of choice was originally casein Later beef fibrin and beef plasma were used, and one preparation was derived from fresh pork (Krishnan *et al*) The hydrolysates most widely used in the United States are four, namely amigen, an enzymatic product of casein parenamine an acid hydrolysate of casein aminosol an acid hydrolysate of fibrin and travamin an enzymatic hydrolysate of beef plasma All of these have had extensive clinical trial and have been shown to be nontoxic and nutritious although there is evidence of some variation in nutritive potentialities depending upon the modes of evaluation

In the preparation of these hydrolysates the trend toward enzymatic hydrolysis has been influenced by the fact that this procedure does not cause the destruction of any of the essential amino acids However the excellent biological value of aminosol indicates that it is possible to employ acid hydrolysis under proper conditions and still avoid a too great injury to essential amino acids But because enzymatic hydrolysis is always incomplete a peptide residue remains in which as much as 45 to 50 per cent of the amino acid nitrogen may be in 'bound form As will be discussed shortly the poorer utilization at times of some of these types of hydrolysate has presented a problem of considerable interest and importance More complete hydrolysis by means of acid obviates this difficulty but presents the danger that if carried too far it may lead to the destruction of certain essential amino acids particularly tryptophan and hence make the hydrolysate nutritionally valueless until it is refortified by the addition of tryptophan thus adding to the cost of manufacture In several of these preparations the high content of glutamic and aspartic acids has tended to cause nausea and vomiting when they are injected intravenously In an attempt to correct this difficulty Merck and Company have prepared an hydrolysate in which these two amino acids are replaced by glycine It has been shown that this preparation can be injected intravenously with little tendency for it to induce nausea but it is not yet available for extensive clinical use A few attempts have been made to prepare amino acid solutions from synthetic amino acids but the results have not been promising both because of the high cost and because of the large amounts of non utilizable unnatural forms of amino acids present in such solutions

Practically all of the hydrolysates now available when properly used are non antigenic and non pyrogenic, and all have been shown to produce positive nitrogen balance in lower animals and man under appropriate conditions of testing Therefore they are metabolic adjuvants of considerable nutritional

that "peptides derived from fibrin may be more efficiently utilized than those of the casein hydrolysate " Thus even though in both instances the peptides were less readily utilized by the tissues than were free amino acids those in a partial hydrolysate of fibrin were not excreted into the urine to as great an extent as were those from an enzymatic hydrolysate of casein

Christensen has compared the fate of the peptides in two protein hydrolysates, one of fibrin and the other of beef plasma when injected into human subjects (normal and convalescent) The acid hydrolysate of fibrin contained only about 4 per cent of amino acids in the ' bound form In this preparation "the loss of peptides after infusion was found to represent about one third of the small amount of peptide nitrogen injected " In contrast an enzymatic hydrolysate of beef plasma contained 46 8 per cent of amino acid nitrogen in the "bound" form After infusion of this preparation, from 31 to 32 per cent of these bound amino acids were lost in the urine Moreover, following injection of the acid hydrolysate of fibrin there was no detectable increase in bound amino acids in the blood plasma and only a minimal wastage in the urine whereas with the enzymatic hydrolysate of beef plasma the peptide nitrogen concentration of the plasma rose as high as 9 2 milligrams per 100 ml He concluded that the peptides infused seem to fall sharply into two classes one utilized and the other not, with few if any intermediate slowly utilized peptides '

Essentially similar conclusions were reached by Silber and Porter in experiments in dogs after the intravenous administration of six different protein hydrolysates and amino acid mixtures The solutions were injected in amounts of 120 milligrams of nitrogen per kilogram per day and the urines were assayed for alpha amino nitrogen by the ninhydrin method before and after acid hydrolysis They found in general that ' the loss of peptides in the urine was greater than the loss of free amino acids and that the biological value of most of the preparations was about 25 per cent less when administered intravenously than when given orally This finding they thought could be "correlated with the urinary loss of approximately 15 or 20 per cent of the infused amino acids and peptides A four fold increase in the rate of infusion led to an approximately 60 per cent decrease in the biological value or nitrogen retention Thus in agreement with Christensen and associates they concluded that the amino acids of certain preparations are more readily excreted unchanged whereas those of other preparations are more readily deaminated so that more of the nitrogen is lost as urea and ammonia nitrogen " They inferred that the spillage of amino acids may be related to biological value in view of the observations of Pearce Sauberlich and Baumann that the feeding to mice of diets containing incomplete proteins led to the excretion on the average of about 24 per cent of the ingested amino acids in the urine in microbiologically available form whereas when complete proteins were fed the average excretion was about 3 per cent With both types of proteins moreover the peptide content of the urines was approximately the same

Silber and Porter also concluded that amino acids were better retained by the tissues than were peptides and that the latter may be unavailable to the tissues because either of size or composition For example one hydrolysate high in peptides and of good nutritional value when given orally was of poor nutritional value when injected intravenously with a loss by way of the urine of 45 per cent of its peptides and 12 per cent of its amino acids They emphasize the fact

amino acid limiting action Too often however, the refusal of acceptance is ascribed to a disagreeable taste which the rat presumably can sense and the real cause of the nutritive inferiority of the hydrolysate is ignored For example, we have demonstrated a considerable degree of variation in the nutritive potentialities of four hydrolysates by the Rat Repletion Method (Cannon, Frazier and Hughes) and that in the case of an acid hydrolysate of casein its nutritive inferiority could be easily corrected merely by the addition to it of a mixture of essential amino acids or even by concentration *in vacuo* We have found, also, that these hydrolysates can be injected subcutaneously into protein depleted rats under comparable conditions of nitrogen intake while at the same time giving the animals by mouth equal intakes of calories vitamins and salts Under these conditions of testing good protein repletion ensues which varies considerably however among the four hydrolysates tested It should be emphasized nevertheless that all four preparations induced a reasonably good protein repletion, indicating that all contained all the essential amino acids necessary for the rat and in an assimilable form for parenteral utilization All of these preparations have also been shown by others to accomplish nitrogen equilibrium in man under proper conditions of testing The only question at issue, therefore, is whether the ones responding less favorably under these conditions of evaluation might not be made as nutritionally potent as the two better ones

The principal point of controversy with respect to the evaluation of an hydrolysate centers around the question of the propriety of using an oral method of assay of an hydrolysate which has been designed for intravenous use in man Obviously all that an oral method can demonstrate is whether or not all the essential amino acids are present in the hydrolysate in reasonable amounts it cannot differentiate, however between those present in free or bound form Nevertheless hydrolysates differ considerably in peptide content depending upon the mode of hydrolysis and although hydrolysates with a high peptide content may be nutritionally of high quality when assayed by an oral method they may be of low biological value when administered parenterally due to the inability of the tissues to utilize peptides efficiently

The importance of the so called 'peptide problem has been stressed particularly by Christensen and his associates who have reported finding a greater urinary excretion of peptides following intravenous injection of partial hydrolysates derived either from casein or fibrin and a greater persistence of peptides than of free amino acids in the blood plasma Evidently under these conditions the free amino acids were better utilized by the tissues than were the peptides When Christensen Lynch and Powers injected an enzymatic hydrolysate intravenously into young men they observed a peptidemia for from three to six hours with a loss of peptides in the urine They concluded therefore that because the tissues exhibited a limited ability to utilize peptides peptides do not contribute nutritional advantage to the preparation' They found moreover that approximately one third of the alpha amino acids were in bound form and *were not* well utilized by the tissues Christensen Lynch Decker and Powers have also demonstrated that the nutritive effects may depend upon the type of peptide For example a partial hydrolysate of fibrin much richer in peptides than an enzymatic hydrolysate of casein and containing approximately two thirds of its amino acids in a conjugated state had a high biological value following intravenous administration in dogs They concluded from these observations

of the blood reached a concentration of 10 milligrams per 100 ml or higher Hecht using the same type of hydrolysate concluded that the rapidity of rise of amino acid levels and not the actual levels themselves were responsible for the side effects

A more detailed study of this problem in human subjects was reported by Smythe, Lesichak and Levey They administered several types of hydrolysate intravenously at different rates of injection and found no relationship between rate of injection and degree of anorexia In more than a hundred subjects tested, the lowest incidence of ill effects was obtained in those injected with the hydrolysate devoid of glutamic and aspartic acids They concluded, therefore, that "the incidence of nausea and vomiting appears to depend on the amino acid composition of the preparation used rather than on the average rate of administration or on the plasma amino acid nitrogen" They found moreover when administering an hydrolysate lacking in glutamic and aspartic acids, that no plasma amino acid nitrogen level observed caused nausea and vomiting uniformly to occur, whereas with digests of casein the increased incidence of nausea was usually associated with high amino acid nitrogen levels

Levey, Harroun and Smythe also determined the free glutamic acid content of the serum of patients receiving hydrolysates intravenously and found a good correlation between these levels and the per cent of subjects who either became nauseated or vomited (57 human subjects) About one half of the subjects became nauseated or vomited when the blood free glutamic acid concentration reached 12 to 15 milligrams per 100 ml, and above these levels the incidence of ill effects became correspondingly greater Paradoxically, however, Messinger has also stressed the fact that at times "solutions of amino acids given intravenously appear to be of value in relieving intractable anorexia" Here again the conclusion must be that more observations will be necessary before the problem can be solved

THE AMINO ACID-SUGAR REACTION OF MAILLARD IN RELATION TO THE UTILIZATION OF PROTEIN HYDROLYSATES

Another important problem with respect to the utilization of protein hydrolysates *viz* that of heat injury due to the Maillard Reaction has arisen because of the current custom of marketing the hydrolysates in glucose solution in order to ensure a greater caloric intake In consequence the problem of sterilization and storage of the hydrolysates has become of greater importance In this reaction more commonly known as the browning reaction a union between sugar molecules and certain essential amino acids leads to a combination which is resistant to bodily enzymes An hydrolysate so affected may be administered in a form in which certain essential amino acids are not readily available for nutritional use Thus Friedman and Kline have reported that autoclaving of protein hydrolysates in the presence of 5 per cent glucose causes some browning and some deterioration of the nutritive quality of the hydrolysate They found also that glucose may combine with several of the essential amino acids, including histidine tryptophan threonine phenylalanine lysine and methionine They pointed out moreover that the amino acid sugar complex may be more readily

therefore that the loss of essential amino acids in peptides accounts for the low biological value of an hydrolysate which is excellent when assayed by an oral method For this reason, all hydrolysates must be assayed parenterally and finally in man

Silber, Seeler and Howe also have emphasized that the utilization of protein hydrolysates depends not only upon the manner of administration, but also upon rates of infusion Thus when they injected the Merck's Amino Acid Solution intravenously into normal dogs at a rapid rate of 220 milligrams of nitrogen per kilogram of body weight, the urinary loss of nitrogen was 13 per cent, whereas when the injection rate was about one sixth of this, only 4 per cent appeared in the urine Thus a rapid rate of injection caused a net loss of urinary nitrogen approximating 9 per cent Furthermore when they injected a racemic mixture of amino acids at a rapid rate, the loss of urinary nitrogen amounted to 23 per cent, in comparison to an 11 per cent loss when the amino acids were injected slowly

In contrast to these observations Allison and associates tested three hydrolysates of casein which varied from 18 per cent to 60 per cent degree of hydrolysis As measured by the K index in dogs they found nitrogen retention practically identical for all three preparations and they concluded that at least a part of the peptides of casein as well as the free amino acids may be utilized to build up the nitrogen stores of the body "

It is obvious that more information is needed before a final opinion can be reached The tissue peptidase content may vary in different species and in individuals of the same species under varying physiological and pathological states However at least enough information is available to suggest that as complete hydrolysis as possible would seem to be advisable provided that in so doing, no serious harm is done to essential amino acids

THE PROBLEM OF ANOREXIA NAUSEA AND VOMITING FOLLOWING THE INTRAVENOUS INJECTION OF PROTEIN HYDROLYSATES

Objection to the intravenous use of protein hydrolysates arises from the fact that some preparations cause nausea vomiting and depression of appetite As Smythe and associates have emphasized a preparation to be acceptable must not interfere with the patient s daily food intake Nonetheless in a study in eight healthy adult males they found considerable variability in this respect For example although an enzymatic hydrolysate of casein caused but slight depression of appetite whether given orally or intravenously an acid hydrolysate of casein "consistently produced a marked depression in the voluntary food consumption during and following intravenous administration On the contrary the injection of an amino acid solution prepared by omission of glutamic and aspartic acids and with substitution of glycine caused no depression of appetite even when injected intravenously at exceedingly rapid rates

This influence of these two dicarboxylic amino acids upon appetite has aroused interest since Madden and associates in 1945 reported that they tended to cause violent vomiting in dogs Hoffman *et al* using an acid hydrolysate of casein observed furthermore, that the nausea occurred when the amino acid content

dietary essentials parenterally under controlled experimental conditions it should also be possible to assess the determining roles of each in their relation to tissue protein synthesis Once that has been accomplished the nutritional evaluation of protein hydrolysates should become a comparatively simple and accurate procedure

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broken down by microorganisms than by the rat, "thus suggesting that micro biological assay of such a preparation may not reveal its utilizability by the mammalian tissues of the rat and possibly of man"

The brown color is not a complete measure of the nutritive deterioration brought about by this reaction and additional information is urgently needed as to the nature of the chemical reaction which can so markedly impair the nutritive potentiality of a protein hydrolysate Particularly serious is the fact that the reaction is a function of time, so that prolonged storage of such a hydrolysate may render it of considerably less nutritive value at the time it may be used in the treatment of a sick patient Some manufacturers are able to keep this reaction at a minimum by avoidance of heat during sterilization and by other maneuvers, but so long as such hydrolysates are stored for long periods particularly under warm conditions the possibility of a shortened shelf life must be considered as a problem requiring further study Here however, the problem may be happily solved if and when the development of a satisfactory fat emulsion for intravenous use renders the present day dependence upon glucose as a source of calories unnecessary

MODES OF ADMINISTRATION

Protein hydrolysates may be injected by various routes *viz* subcutaneous intravenous intramuscular intrasternal and intraperitoneal Usually however the intravenous route has been preferred particularly with those containing glucose despite the disadvantages of injury to veins, nausea and vomiting and toxic reactions from too rapid infusion and the loss of peptides and amino acids via the kidney For these reasons attempts have been made to inject hydrolysates intramuscularly Thus Weinstein has reported success in administering hydrolysates into the lateral muscles of the thigh in the subfascia lata space and has been able to secure a rapid infusion (within thirty minutes) of a liter of 5 per cent hydrolysate solution without local reactions or failures of absorption He maintains that this method has the advantage of simplicity requires little skill in accomplishment and avoids long periods of immobilization and fixation of the arms It also tends to decrease the incidence of reactions both local and general The more gradual absorption prevents the rapid rise in blood amino acid levels which has been attributed by some to cause nausea and vomiting This method also can be used by the nursing staff thus releasing the pressure on the interns and residents where intravenous methods are depended upon He has been able in this way to administer 3 liters of hydrolysate per day giving it with glucose intravenously in the morning and evening and intramuscularly in the afternoon

From the above discussion the conclusion seems warranted that the more effective clinical use of the protein hydrolysates in parenteral nutrition must await the solution of several perplexing problems Some of these moreover may prove to be of incidental interest once the basic difficulty of caloric inadequacy has been overcome Protein metabolism as it operates in tissue maintenance and reconstruction is a complex dynamic process requiring the coordinated interaction of calories amino acids vitamins enzymes systems and salts and the lack of any one of these nutritive essentials may act to some extent at least as a limiting factor However when it becomes possible to supply simultaneously all the basic

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Chapter

2

MELANOTIC TUMORS OF THE SKIN

By J. A. Cunningham, M.D.

INTRODUCTION

Pigmented lesions of the skin are so common that every adult is estimated to have at least one pigmented mole. Despite this fact, of all the skin tumors pigmented ones are the most often misdiagnosed.[2] The consequences are obvious. Failure to recognize an early melanoma destroys the optimum chance of cure by radical treatment. Erroneous interpretation of junctional changes as stigmata of malignancy results in unnecessary and often mutilating surgical intervention—to say nothing of the profound psychic trauma and expense to the patient.

In recent years intensive study has polished the practical and theoretical facets of this problem and much of the confusion has yielded to the process. Today a clearer concept of the histogenesis of pigmented nevi and malignant melanomas has resulted in sharper histologic criteria for the microscopic differentiation of these lesions. At the same time, practical clinical information has accumulated for the guidance of the practitioner in the day to day diagnosis of the ubiquitous pigmented mole.

HISTOGENESIS

Fundamental to any consideration of this problem is a basic understanding of the two major competitive theories of the origin of pigmented nevi and malignant melanomas. These are the epidermal and neural theories. (The older concepts, namely, the endothelial and chromatophoric, have been virtually abandoned.)

The epidermal protagonists, led by Unna[43] and lately by Allen,[3] contend that the nevus cell takes its origin from the true epidermal cells by a process of gradual differentiation, and that these differentiated cells migrate into the corium by means of the so called *Abtropfung* (or dropping off process). The differentiation of basal or other epidermal cells to melanoblasts is thought to occur *in situ* and to consist of a morphologic alteration with concomitant acquisition of the ability to form the enzyme melanogenase.

The neurogenic theory, first suggested by Soldan[37] in 1899 and ably supported by the painstaking and brilliant investigations of Masson,[21, 23] Foot,[12] Becker[5] and others, is more widely accepted and has received considerable bracing from the experimental biologists.[33, 47] This theory maintains that nevi are derived neoplastically or disontogenetically from two sources primarily—even though all the constituents of the integument may take part.

One of these sources is the intradermal dendritic melanoblast (Fig 1) This cell though constantly situated in the basal layer, is a specialized cell *sui generis* and the only one which gives a positive dopa reaction It is characterized by its arborization, its content of melanogenase and its ability to discharge its secretory product (melanin) into the cells of the malpighian layer as well as into the connective cells of the papillary layer of the dermis The precise origin of these cells whether from the epidermis or neural crest is still obscure The neuralists lean to the concept that they arise in the neural crest and later wander into the epidermis in the same manner as occurs in birds and cold blooded vertebrates Laidlaw and Murray [19] following this line of thought have compared the nevi to the tactile areas of the crocodile, and consider them malformations or tumors in man equivalent to these analogous structures in the lower vertebrates

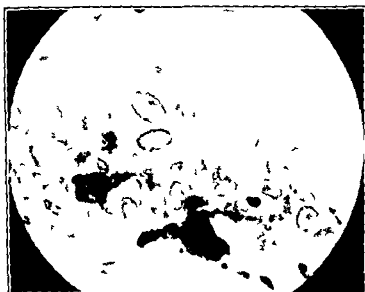


Fig 1 —*Dendritic Melanoblast* Note basal position and the pigmented dendrites extending from cell body This discharge of melanin through dendrites into cells of the malpighian layer is referred to by Masson as cytocrinia

The other important source of the nevus (neurogenic theory) is the proliferation of the Schwannian syncytium around the dermal nerves The melanoblasts drop off into the epidermis and wander down into the upper dermis The Schwann cells proliferate and tend to migrate upwards in the dermis Where they fuse the complicated nevic plexus is seen Support for the participation of the Schwannian syncytium in the development of the nevus is ably brought by Masson[25] in his demonstration that nevus cells have the histologic characteristics of the Schwannian syncytium and in fact differentiate to form structures anatomically almost identical with Wagner Meissner corpuscles He shows further that there is always at least incomplete neurotization of the dermic portion of the nevus

From the foregoing discussion it is quite clear that the problem of the common mole and its less frequently encountered malignant analogue are so closely related that they must be considered together (In the interest of clarity it should be

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millimeters to several centimeters in diameter It may be present at birth or may develop at any later time, often appearing suddenly in crops If a pigmented lesion is found on the palmar surface of the hand plantar aspect of the foot or on the scrotum, it may be assumed that it is a junctional nevus inasmuch as the ordinary intradermal nevus is almost never found at these sites But the junctional nevus may also occur on the skin of practically any part of the body

The name 'junctional nevi' is applied to these tumors because histologically there is proliferation of nevus cells which starts (and is most conspicuous) at the junction of the dermis and epidermis Knobs of clear cells are seen interposed between the basal cells and often higher in the malpighian layer The picture is quite characteristic with clusters of nuclei in what appears to be an empty space and surrounded by typical squamous epithelial elements These clear

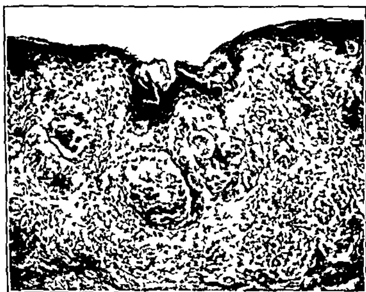


FIG 2 –*Junctional Nevus* The epithelial dyskeratosis marked clear cell (melanoblast) proliferation in basal and junctional areas and lymphocytic infiltration of dermis are quite typical

cells are usually uniform in size and staining reaction with their cytoplasm often containing granules of melanin The seeming lack of cell membrane is a striking histologic feature The peculiar proliferation of nevus cells is also seen in the prolongations of the epidermis about the hair follicles and sebaceous glands Mitotic figures are rarely encountered despite the apparent loss of cohesion between the individual clear cell elements Not infrequently a narrow zone of inflammatory cells—principally lymphocytes—is seen in the dermis immediately beneath the lesion Melanin laden histiocytes (easily confused with invading melanoblasts) may be found in amongst the lymphocytes (Fig 2)

It is important to remember the proclivity of the junctional nevus to show skip areas In checking its margins one should always bear in mind that even though apparently normal epidermis completely surrounds the specimen out to the edges of the surgical excision skip areas of similar change may lie just beyond Undoubtedly this trait accounts for some of the recurrences of nevi in excisional

pointed out that the term "nevus" used synonymously with the term "mole" is used in a restricted sense to indicate those tumors made up essentially of nevus cells, and not in the broader sense of the dermatologists to indicate any congenital skin blemish. Also, the term 'melanoma' is here used to designate only the malignant melanoma.)

PIGMENTED NEVI

Most published studies of melanomas report, in over 50 per cent of the cases, the previous history of a pigmented nevus at the site where a melanoma has occurred (see Table I). That the relationship is a real one is evidenced by those instances where residual benign nevus cells can be seen at the margin of a frankly malignant melanoma. In view of these findings the role of the nevus as a melanoma precursor is worthy of consideration.

TABLE I.—ORIGIN OF MELANOMAS IN PRE-EXISTING MOLES

Authors	*Total Number of Melanomas*	*Percentage with Pre-existing Mole*
Wright	94	49
Daland & Holmes	128	44.6
Pack	862	50
Ackerman	75	61
Driver & McVicar	60	80
Total	1219	56.9

Until recently any approach to this type of investigation was hindered by an unwieldy and complex terminology. One of the products of the competition between the neural and epidermal schools has been a simplified useful terminology for the many varieties of nevi. This has been one of the major contributions of the epidermalists. In their studies they divide nevi into four groups, namely (1) junctional nevus (dermal epidermal nevus or marginal nevus), (2) intradermal nevus (the common mole or neuronevus), (3) the compound nevus, and (4) the blue nevus (Jadassohn Tieche). These designations are recommended for their practical value, for they allow the slotting of the histologic variants for closer study.

At the same time it should be pointed out that the neuralists feel there is little sense in breaking down these tumors into separate groups since those developing in the deep layers will assume the complicated neurofibromatoid appearance while those arising beneath the epidermis will be cellular and alveolar. The type of cells seen will vary as it inclines now toward those of the Merkel-Ranvier group, now toward those of the Meissner corpuscle.

The question naturally arises, if the nevus is considered to be a potential precursor of the melanoma, should not all nevi be removed? As this is patently impracticable, the next question is, which nevi are the dangerous ones and how can they be recognized? *Unfortunately no clear cut answer can be given*, but certain observations are worthy of thought and give at least a partial answer. The first step, however, should be toward a clear visualization of the gross and microscopic features of the four types of nevi.

1. **The Junctional Nevus.**—Clinically, the lesion is a smooth, hairless brown to black macule which is flat or slightly elevated and varies from a few

sweat glands into the deep dermis Mitotic activity however, is seldom seen, and although the nevus cells at various levels are strikingly different in appearance there is no appreciable variation in cell size or staining reaction (Fig 3)

It is this type of nevus that is thought to undergo rarely, if ever malignant change

3 **The Compound Nevus** —This tumor derives its name from the fact that it is a combination of the junctional nevus and the intradermal nevus Most nevi in children are of this type—in fact 98 per cent of 50 nevi examined by Spitz[15] showed this structure This is of particular interest in view of the infrequent occurrence of malignant melanomas prior to puberty Of all the nevi in adults 12 per cent fall into this category (Fig 4)

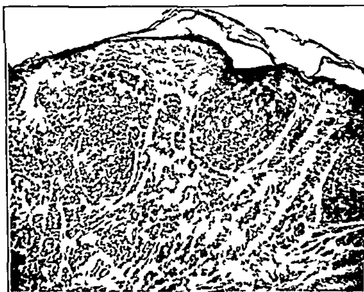


FIG 4 —*Compound Nevus* This shows combination of junctional activity and large clusters of small nevic cells in superficial and deep dermis

Because of its junctional component the compound nevus is also considered to be a melanoma precursor The occurrence of a melanoma on a long standing nevus is thought to be accounted for by this type of lesion

4 **The Blue Nevus** —This nevus differs fundamentally from those previously described in that the type cell from which it is derived though truly a melanoblast showing the characteristic dopa stain is thought to be mesodermal in origin It is a smooth and hairless growth flat or slightly elevated sharply circumscribed round or oval and blue gray or blue black in color It usually occurs on the face buttocks or dorsum of the hands and feet and is made up of cells identical with those seen in the sacral Mongolian spot of human beings and in the corium of certain vertebrates

Histologically it is made up of greatly elongated fusiform cells with bipolar and often branching processes and with their long axis parallel to the epidermis These are situated in the lower and middle third of the corium occasionally extending upward close to the epidermis and downward into the subcutaneous

scars, and (more important still) accounts for the development of a frank melanoma in the scar of a tumor perhaps *correctly evaluated as a junctional nevus* at the time of its removal

It is this junctional nevus with its restless appearance and the apparent loss of *cohesion between clear cell elements that has often been erroneously* diagnosed as a malignant melanoma It is also the lesion which the epidermalists hold to be the true precursor of the malignant melanoma—on the basis of which belief *they strongly* recommend the removal of pigmented *foci* from such areas as the scrotum and the plantar and palmar aspects of the extremities

2 **The Intradermal Nevus** —This is the 'common mole' It can occur anywhere on the skin surface though it is rare in the regions previously noted Its color varies from pink to brown Most often it is a papillary warty growth, but it may be flat Usually there are a few fine hairs projecting from its surface



Fig 3 — Intradermal Nevus (Common Mole) Atrophic epidermis without junction change and clusters of small nevic cell in superficial and deep dermis are here shown The foliated lamina caricaturing Wagner Meissner corpuscles are not seen in the picture

Infection of the hair follicles accounts for some melanoma scares The histologic picture is complex with practically the entire skin and its appendages involved in the process The epidermis is *markedly dyskeratotic showing hyperkeratosis* acanthosis and parakeratosis as well as atrophy in varying combinations The upper dermis is infiltrated by clusters of nevus cells arranged in irregular epithelioid or alveolar pattern *The mid dermis and lower dermis show the complicated* nevic plexus It is at this *level that the nevus cells usually become more* fusiform—often growing around and in close apposition to sweat glands Here the well recognized leaflike lamina *(lames foliaces) are seen caricaturing the* Wagner Meissner corpuscles

This lesion is never encapsulated in fact to the uninitiated it often presents the appearance of an infiltrating growth particularly where it *extends around the*

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It is this type of nevus that is thought to undergo rarely, if ever, malignant change

3 **The Compound Nevus** —This tumor derives its name from the fact that it is a combination of the junctional nevus and the intradermal nevus Most nevi in children are of this type—in fact 98 per cent of 50 nevi examined by Spitz[31] showed this structure This is of particular interest in view of the infrequent occurrence of malignant melanomas prior to puberty Of all the nevi in adults 12 per cent fall into this category[2] (Fig 4)

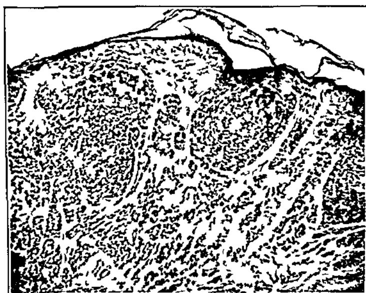


FIG 4 —*Compound Nevus* This shows combination of junctional activity and large clusters of small nevic cells in superficial and deep dermis

Because of its junctional component the compound nevus is also considered to be a melanoma precursor The occurrence of a melanoma on a long standing nevus is thought to be accounted for by this type of lesion

4 **The Blue Nevus** —This nevus differs fundamentally from those previously described in that the type cell from which it is derived though truly a melanoblast showing the characteristic dopa stain is thought to be mesodermal in origin It is a smooth and hairless growth flat or slightly elevated sharply circumscribed round or oval and blue gray or blue black in color It usually occurs on the face buttocks or dorsum of the hands and feet and is made up of cells identical with those seen in the sacral Mongolian spot of human beings and in the corium of certain vertebrates

Histologically it is made up of greatly elongated fusiform cells with bipolar and often branching processes and with their long axis parallel to the epidermis These are situated in the lower and middle third of the corium occasionally extending upward close to the epidermis and downward into the subcutaneous

fat They are arranged in peculiar, often disconnected, fascicles which give an erroneous impression of invasion of lymphatic vessels The tumor is heavily pigmented, with abundant melanin in both the melanoblasts and adjacent chromatophores This pigmentation is often so dense that it is impossible to separate the chromatophores from the enzyme containing cell elements Mitotic figures are rarely seen Upon close inspection after depigmentation the individual cell components are found to be strikingly uniform in size, shape and staining reaction (Fig 5)

This type nevus is essentially benign *Its rare malignant transformation is* thought to account for the occasional true melanosarcoma



FIG 5 —*Blue Nevus (Jadassohn Tieche)* Note intact unchanged epidermis In lower and *middle third of dermis long fusiform melanoblasts heavily pigmented are readily seen with* their long axis parallel to epidermis

SUGGESTIONS FOR CLINICAL HANDLING OF PIGMENTED NEVI

Clinical examination alone will not always permit the separation and identification of the potentially dangerous nevi but certain observations have given rise to some practical suggestions for the handling of pigmented lesions These are worthy of discussion

To begin with if as has been indicated the junctional and compound nevi are the melanoma precursors, then it follows that they should be removed when possible From this comes the logical recommendation that *all* pigmented lesions on the palms of the hands the soles of the feet and on the scrotum be removed since almost all of these are of the junction type

Another useful observation is the rarity of ulceration in nevi and its contrasting frequency in melanomas This was confirmed by a study of 100 benign nevi and 100 primary melanomas from our files as well as by similar studies by others [48]

The inference to be drawn here is that, while all ulcerated pigmented lesions are not malignant they are at least highly suspect and should be promptly removed and histologically examined Unfortunately, ulceration is not a sign of *early* malignant change, and not infrequently when observed at least microscopic deposits of tumor cells are already present in the lymph nodes

Also of value is the observation that melanomas in contrast to nevi, often have irregular pigment borders Hence, a pigmented lesion with an irregular border (and more particularly if it has irregular fingerlike projections or shows apparently disconnected small satellite foci) should be considered malignant until proved otherwise

A further practical suggestion is that all nevi likely to be subjected to chronic irritation be removed Although the role of trauma in the cancerization of pre existing benign nevi is debatable and while real proof is lacking there is enough suggestive evidence to justify this recommendation

Finally in reaching a decision concerning the removal of a nevus certain endocrine factors should be borne in mind particularly as the effect of hormonal stimulation on pigmented nevi and melanomas has been well recognized We need only recall the pigmentary changes (chloasma) of the face, nipples and abdomen of pregnant women to appreciate the role of hormones in pigment metabolism A further indication of this relationship is the increase in size and pigmentation of pre-existing nevi and the appearance of apparently new crops of these tumors at puberty Add to this the remarkable biologic difference in behavior of the essentially benign prepubertal melanoma and its histologic twin the adult melanoma and the importance of hormone factors becomes very obvious In line with this Pack[29] has suggested that every prenatal examination of the mother should include a survey of her pigmented moles, and those subject to irritation as well as any situated on the genitals or feet, should be removed and histologically examined He has also recommended that potentially dangerous nevi in children be removed prior to the onset of puberty

In summary the surgical removal and histologic examination of the following lesions is recommended

1 All pigmented lesions on the soles of the feet palms of the hands and on the scrotum
2 All ulcerated pigmented lesions
3 Those pigmented lesions subject to chronic irritation by reason of their anatomic location
4 Pigmented lesions with irregular borders pigmented fingerlike projections or with accompanying satellitosis
5 Any suspect nevi in children prior to puberty
6 Any pigmented lesion on the genitals and feet as well as those subject to irritation of all pregnant women in the early prenatal period

SKIN TUMORS OFTEN CONFUSED WITH NEVI AND MELANOMAS

1 **Seborrheic Keratosis** (*Verruca Senilis Senile Keratosis*) — Because this warty growth often shows an increased melanin content and accumulation of considerable debris on its surface it has been mistaken clinically for a nevus or

melanoma The papillary acanthosis, with fusion of the rete pegs and the presence of pseudocysts filled with keratin debris plus the absence of nevus cells makes its histologic differentiation quite simple

2 **Pigmented Basal Cell Carcinoma** —At times it is actually impossible to differentiate this tumor from malignant melanoma by clinical methods Often it is heavily pigmented (Fig 6) surface ulceration is not uncommon and quite frequently it is found in anatomical situations where melanomas are not unusual Histologically the lesion presents no difficulty in diagnosis



Fig 6 —*Pigmented Basal Cell Carcinoma* Heavy pigmentation is seen in center of larger downward invading epithelial bud This tumor is often confusing clinically but not histologically

3 **Sclerosing Hemangioma** This tumor may not be easy to classify clinically because of heavy pigmentation—and here again ulceration is often present Histologically the diagnosis is made without difficulty Although it has been mistaken for the fasciculated malignant melanoma an iron stain should readily distinguish the two types of pigment (Fig 7)

4 **Dermatofibrosarcoma Protuberans** — Seldom densely pigmented this lesion has been confused with the fasciculate amelanotic melanoma by both clinician and pathologist The histologic picture is more suggestive of a fibrosarcoma and does not have the swirling streaming characteristic of the melanoma (Fig 8) (Furthermore melanin containing cells can almost always be found in a melanoma if a thorough search is made) It is of importance to avoid this error of diagnosis since this tumor is usually only locally malignant and the treatment and prognosis are quite different from that of the malignant melanoma



FIG 7 —*Sclerosing Hemangioma* Note curly nature of the fusiform cell proliferation Pigment is hemosiderin

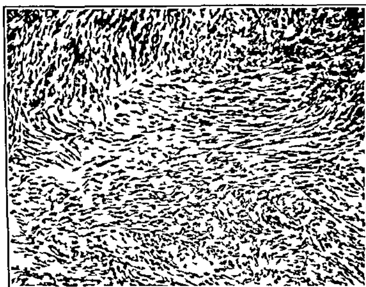


FIG 8 —*Dermatofibrosarcoma Protuberans* The interdigitating long spindle shaped cells lack the swirling streaming characteristics of the fasciculate melanoma

THE PREPUBERTAL MELANOMAS

Until recent years certain features of the nevi of childhood particularly junctional change were considered stigmata of malignancy As this junctional change is present in 98 per cent of nevi in children [38] it is understandable that many errors occurred With the fuller realization that junctional proliferation does not in itself indicate melanoblastoma this source of error was largely eliminated

It soon became apparent, however, that certain tumors in children present all the clinical and microscopic features of the malignant melanoma of the adult (Fig 9) Follow up studies showed that these neoplasms, diagnosed as melanomas by competent and experienced pathologists, rarely behaved like malignant tumors This disparity of biologic behavior despite the apparent identity of morphologic pattern is a matter of fundamental importance from the therapeutic and prognostic viewpoint

Pack *et al* [30] reported 15 cases of prepubertal melanomas (one of which metastasized) and all survived indefinitely These investigators are of the opinion that hormonal factors, originating in the pituitary gonads, and adrenal cortex, play some role in changing the biologic behavior of this tumor



FIG 9 —*Prepubertal Melanoma* Epidermis is ulcerated Cells are large fusiform basophilic show numerous mitotic figures and extend in streamers to deep dermis

In 1948 Spitz[38] went somewhat further in her report of 13 cases of melanomas in children who had not reached puberty Only one of these ended fatally and this was the only one in the series that showed metastasis The 12 cases without metastasis were treated by simple local excision Although Spitz observed unusual giant cells in 8 of the 13 cases she is of the opinion that histologic differentiation of juvenile and adult melanomas cannot be made with certainty

Numerous other reports of juvenile melanomas show a pattern of behavior that is much more benign than the usual adult melanoma but still other cases have been reported in which the progression of the disease in the prepubertal patient has been essentially similar to that in the adult [3, 4]

This prepubertal melanoma can be compared to a live cartridge in the skin which needs only the trigger effect of the profound hormonal stimulus of puberty

to set off the explosion. That hormonal stimulation is not necessary to the sustained growth of the melanoma, once its biologic potential has been released, can be seen in the apparent lack of effect of castration on the progress of the tumor. Further proof of this thesis is found in the fact that melanoma cells transmitted to the fetus via the placenta show no evident growth restraints long after the hormonal stimulation from the mother ceases.

Certain conclusions follow these observations:

(a) Age of the patient is of extreme importance in evaluating the pigmented tumor.

(b) All suspect nevi should be prophylactically removed prior to puberty.

(c) Aside from the occasional "sport" melanoma (which behaves like its malignant elder) most pigmented tumors in children are essentially benign and can be so treated.

THE MALIGNANT MELANOMA

Of all skin tumors the melanoma is certainly the most dangerous. It is an interesting paradox that this readily visualized tumor which pinpoints itself by virtue of its pigmentation has already metastasized in over 40 per cent of the cases on admission to the hospital.

Origin.—These tumors either arise on pre-existing nevi (see Table I) or appear to arise *de novo*. The pre-existing nevus is usually of the smooth, hairless, slightly elevated type, and the change in its character is often indicated clinically by increased size, deepening of pigmentation, or by ulceration. When it seems to arise *de novo* the possibility of a small flat intradermal type of junctional nevus or lentigo having preceded it cannot usually be ruled out.

Incidence.—The over-all population incidence has been estimated by McDonald as 1.8 per 100,000. Figures on the ratio of melanomas to other skin tumors are quite variable. Sylven[40] found that melanomas constitute 7 per cent of the skin cancers at the *Radiumhemmet*. Ackerman,[1] on the other hand, found that they totaled only 2.9 per cent of the skin cancers in his series.

Sites of Occurrence.—From the accompanying table which represents the distribution found in 960 cases (see Table II) it is readily seen that the tumor can occur on any skin surface but is found in highest concentration in the region of the head and neck and on the lower extremities.

TABLE II.—SITES OF OCCURRENCE OF 960 MELANOMAS

Site	*Wright (109 cases)*	*Pack (851 cases)*
Head & Neck	26%	23.1%
Trunk	28%	17.9%
Upper Extremities	13%	13.7%
Lower Extremities	27%	27%
Other Sites	6%	18.3%
	(Eye not included)	(Eye included) 8.6%

Sex Distribution.—McDonald, in reporting 349 cases, showed that 49.3 per cent occurred in males and 50.7 per cent in females. This coincides with

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Two basic cell types are seen in the malignant melanoma the epithelioid and the fasciculate or sarcomatoid Not infrequently both types are found in the same tumor

If the epithelioid elements predominate the low power design is either that of melanoma cells clustered in nevoid nests (Fig 10) (in which case the name nevocarcinoma is sometimes applied) or of cells arranged in irregular alveolar patterns (Fig 11) These epithelioid elements vary from small to quite large The *small* cells are usually not much larger than a nevus cell, and have a dark, hyperchromic nucleus and a pale staining often vacuolated, cytoplasm Sometimes they are confused with reticulum cells, particularly when seen as metastatic deposits *in a lymph node* *The large epithelioid elements* show a hyperchromic nucleus often with a prominent eosinophilic nucleolus a pale pink staining, acidophilic cytoplasm with or without pigment granules These large cell ele-

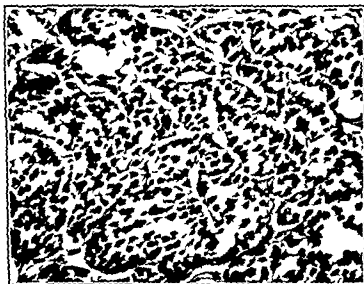


Fig 11 — *Malignant Melanoma (Alveolar Type)* Epithelioid cell elements are arranged in irregular alveolar patterns There is still some suggestion of nevic structure but the cells are larger the arrangement more disorganized

ments often bear a superficial resemblance to ganglion cells and on occasion have been mimicked by undifferentiated squamous cell carcinoma (Fig 12)

The sarcomatoid or fasciculate melanoma is made up of spindle shaped cells with bipolar processes and a central basophilic ovoid nucleus These cells are arranged in fascicles or swirling bundles in a manner closely resembling a neurofibrosarcoma (Fig 13)

Pigmentation is extremely variable irrespective of the cell type encountered In some tumors pigment is found with great difficulty and in these the dopa stain is invaluable Unfortunately too often the diagnosis is not suspected until prolonged fixation has occurred with destruction of the intracellular enzyme Staining the fixed sections with silver has been recommended for demonstrating small amounts of pigment but in my own hands this procedure has been of little value There are other melanomas in which the pigment deposition is so heavy

the findings in other series and indicates that there is no significant difference in incidence between the sexes

Age Distribution —As has been previously pointed out, malignant melanoma is rare before puberty A large majority of the cases are found in the thirty five to seventy age group[32]—in this respect corresponding with other malignant tumors

Race Factor —It is generally conceded that pigmented nevi and melanomas are not commonly found in the darker races The ratio of incidence for melanomas in white and colored individuals is estimated as 3 or 4 to 1 [16] (An interesting biologic corollary to this is the observation that melanomas are more frequent in light colored horses) Another peculiarity of this tumor in the colored races is its predilection for the soles of the feet the nail bed, and the oral mucosa—regions not usually deeply pigmented



FIG 10 — *Malignant Melanoma (Nevocarcinoma)* Malignant melanoblasts are small and arranged in nevic clusters

A theory has been advanced to explain the uncommon occurrence of the melanoma in the Negro and its strange anatomic distribution This theory postulates that the physiologic function of pigment formation is under better control in heavily pigmented skin and hence the cells capable of producing pigment are less prone to unrestrained growth In concert with this idea is the clinical observation that malignant melanomas are found most often in blond individuals with pale skin

Histology of the Melanoma —Despite its characteristic diversity of histologic structure the full blown melanoma presents little diagnostic difficulty to the experienced histopathologist but unfortunately doubtful and transitional melanomas are not rare These are the tumors which often require a ' nicety of judgement to resolve the issue (The problem of the doubtful melanoma will be discussed under a separate heading p 43)

DOUBTFUL MELANOMAS

These are the cases which present the greatest diagnostic problem to the experienced pathologist. That they exist, and are in fact not uncommon, is not widely appreciated. The necessity of studying these borderline lesions is obvious, yet surprisingly little work has been done thus far. Histologically, they are not simple variants of nevi, and at the same time they are not outright melanomas.[18, 48] They are tumors in which the cell components lie somewhere *between* the nevus cell and the melanoma cell.

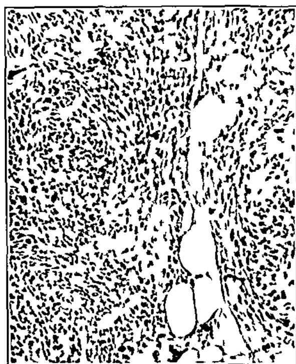


FIG. 14.—*Borderline or Doubtful Melanoma.* Note deep penetration of fat and the more important basophilia, cell variation and disorderly arrangement of pigmented cell elements. Mitoses were found in this case. Local excision resulted in eight year cure.

In some instances there is excessive deep penetration by enlarged, hyperchromic, pigmented fusiform elements—this penetration extending around the sweat glands and into the adjacent fat (Fig. 14). Not infrequently these altered cells show mitotic activity. In essentials what is seen is an exaggeration of the penetration that is often found around the sweat glands in the ordinary intradermal nevus, but in the doubtful cases the cell components show much more cell variation, basophilia, and other cell features suggestive of an aggressive neoplasm. Sometimes the change is limited to the junctional zone in the epidermis (Fig. 15), in which cases instead of ordinary nevus cells in the junctional area, the cells are larger, show less cell cohesion, bizarre and often enlarged hyperchromic nuclei, and occasional mitoses. Frequently there is a zone of lymphocytes and an increased number of chromatophores in the dermis. Everyone experienced in

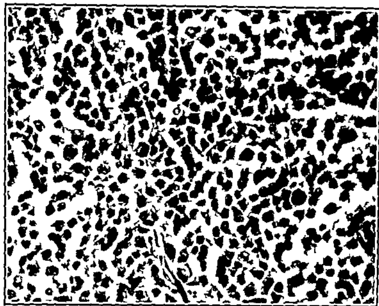


FIG 12 — *Malignant Melanoma* Note large size of melanoblasts the loss of cell cohesion Resemblance to ganglion cells is best seen in H and E sections

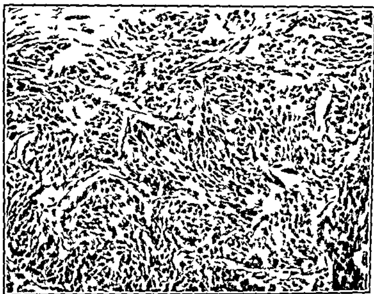


FIG 13 — *Malignant Melanoma (Fasciculate Type)* Resemblance of this type of melanoma to a neurosarcoma is evident

that the underlying tumor cells can scarcely be visualized Depigmentation of such tumors makes it possible to more adequately study cell detail

All of these varieties of melanomas are further characterized by the usual histologic stigmata of malignancy namely increased cell size variation in cell size increased mitotic activity loss of cell cohesion and local and lymphatic invasion Another important and *constant* feature of the primary melanoma is involvement of the epidermis in a bizarre type of junctional activity [3]

THE MALIGNANT MELANOMA AND PREGNANCY

Because of the unusual rapidity with which the melanoma grows and metastasizes in conjunction with pregnancy, prime consideration must be given to prophylaxis. For this reason it was strongly recommended earlier in this discussion that all potentially dangerous pigmented nevi be removed prior to pregnancy or early in the prenatal period. If histologic examination shows any to be melanomatous, radical surgical treatment following accepted standards should be undertaken immediately.

Questions have arisen as to the value of therapeutic abortion and hormonal therapy as adjuncts to the surgical procedure. With regard to the first, recent studies[9] show that abortion is not indicated, since all but a rare few of the children born to melanoma bearing mothers are normal and the interruption does not in any way seem to alter the progress of the disease. On the other hand, it is considered wise to advise patients with treated melanomas to avoid pregnancy for at least three years. The question of the advisability of hormone therapy has been answered by clinical experiments.[9] These have included surgical castration, large doses of testosterone, pituitary irradiation—even bilateral adrenalectomy with cortin administration. Without exception the record is one of complete failure.

METASTASIS

The primary route of metastasis is through the lymphatics. The spread is either by embolism or by permeation through the lymphatic trunks or subcutaneous lymphatic plexuses to the lymph nodes. Regional lymph node involvement is a very common occurrence.

As a rule, metastasis of this type takes place within three years. Taylor and Nathanson[41] showed in their study of melanomas with metastases that lymph node involvement occurred in 57 per cent of the cases within a year, 70 per cent within two years, and 83 per cent within three years. Intra-ocular melanomas are notorious for delayed metastasis, but the cutaneous tumors also have a disquieting tendency to reappear in general dissemination years after apparent cure. Wilbur and Hartman[46] point out that metastasis may show up after ten to thirty years. This feature of the melanoma is mirrored in the marked drop in salvage rates between the five and ten year intervals as reported by most authors. For this reason any evaluation of five year survival rates must be guarded.

Statistical studies[41] have been undertaken in an attempt to relate the incidence of lymph node metastasis to such variable factors as race, age and sex of the patient, duration and size of the primary tumor, its anatomic location, histologic type, and previous treatment. Although variations in incidence were noted, they were inconstant and not statistically significant.

The clinical appraisal of metastatic lymph nodes is not reliable. A recent study[41] showed microscopic deposits of tumor in 19 per cent of the nodes which were not palpable, while an equal number of palpable nodes were innocent. As in other tumors there is a greater chance of clinically recognizing involved lymph nodes in the cervical region than in the axillary or inguinal areas. (It should be pointed out here that all pigmented cells seen microscopically in lymph nodes do not represent metastatic melanoma. Hemosiderin and melanin laden macro

surgical pathology has seen this type of lesion, and, even after prolonged study of serial sections, has on occasions been forced so to speak to play it by ear

Wright[43] in an interesting review of 148 melanomas found that 27 cases fell into this doubtful group Of these, 10 occurred in children under twelve years of age, and, since malignant melanoma is rare in that age group could probably be eliminated on that basis The other 17 still remained problems

He found that the lesions occurred most often on the face and lower limbs—these two areas accounting for 85 per cent of the total number It was noted too that all the lesions in the doubtful group were small only one was ulcerated and pigmentation though variable was not prominent A follow up showed all of them to have a long survival period, with no conclusive evidence of malignancy

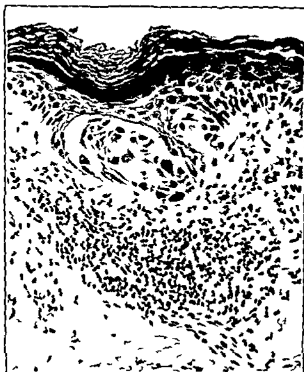


FIG 15 —*Doubtful Melanoma* Epithelial covering is intact There is junctional activity in epidermis but note variation in cell and nuclear size loss of cell cohesion and lymphocytic infiltration in dermis An occasional mitotic figure was found in altered nevic elements

The altered nevus or doubtful melanoma which shows deep penetration of bizarre spindle shaped elements (but *without* associated junctional activity) though disturbing to the oncologist can in most instances be treated by local excision (As a precautionary measure however the patient should be observed for several years)

But the other type of borderline lesion—the one *with* atypical junctional activity—clearly and inexorably raises the question *Is this an early malignant melanoma?* With our present understanding of these doubtful tumors our answer is halting and fallible Further study of this problem is both a challenge and an urgent need

GROUP II CASES WITHOUT CLINICALLY EVIDENT LYMPH METASTASIS

Because of the fallibility of clinical evaluation of lymph node metastasis there is general accord that prophylactic lymph node dissection should be carried out in this second group of cases Of first importance, however, is the radical excision or amputation of the primary tumor This should include a margin of skin 6 to 10 cm wide and will require an immediate skin graft The depth of the excision should extend below the deep lymphatics thus including the underlying fat and fascia

In those instances where the skin melanoma is situated close to the first relay of draining lymph nodes excision and dissection in continuity is recommended [31] This involves the removal of a large strip of skin and fascia from between the primary tumor and the regional nodes in such a manner as to take in the sub cutaneous lymphatic plexus and the deep lymphatic trunks of the area This procedure (designed to eradicate the primary tumor all tumor cells in transit in the lymphatics and in the lymph nodes) cannot be used when the primary is too remote from the draining nodes—as when the melanoma is situated on the foot

In such cases of wide separation radical excision of the primary lesion is carried out then a period of several weeks is allowed to elapse before the prophylactic node dissection is done This delay is recommended by some[31] to permit the interception of lymphatic emboli either already in transit or set free by the manipulation incidental to the operative procedure on the primary In my own opinion, the value of this delay period is questionable particularly in view of the imperfect and transient barrier afforded by the interposing lymph nodes to the continued spread of the tumor It could also be argued that, since there is no assurance that microscopic deposits of tumor are not already in the nodes any delay in resecting them only adds to the patient s jeopardy

GROUP III CASES WITH CLINICAL EVIDENCE OF OPERABLE REGIONAL LYMPH NODE METASTASIS

Cases with obvious lymph node metastasis are best treated by excision and *dissection in continuity* but *as was noted in discussion of Group II* the primary site of the melanoma is often the hand or foot eliminating the possibility of this type of management For such patients there is a choice between two methods of treatment

The first is to radically excise the primary lesion and after an interval of several weeks follow this by radical lymph node dissection of the drainage area But if lymph node involvement is extensive and there is an associated lymph stasis this procedure which leaves behind all the intervening lymphatics offers little hope of a cure It is these patients who frequently show multiple recurrences and metastases in the deep lymphatics and subcutaneous tissues

The second method of management for this group of cases is hip joint disarticu lation with dissection of the deep nodes of the groin when the melanoma is on the foot and the interscapulothoracic amputation with dissection of the lower cervical lymph nodes when one of the upper extremities is involved The

phages are found in a wide variety of conditions and can mislead the unwary.)

Another important route of melanoma metastasis is by way of the blood stream. This is much less frequent than lymphatic spread and is usually a late event. Sometimes the fortuitous invasion of a blood vessel with widespread metastasis occurs early in the disease. In such cases the liver, lungs, heart, spleen, brain, and skin are often the sites of metastatic tumor growth.

An interesting result of blood stream metastasis is the occasional finding of deposits of tumor in the placenta, with transfer of the tumor cells to the fetus. Several such reports have been recently published.[9, 15, 39, 44] One of these describes the case of a twenty five year old woman with a cutaneous melanoma on the inner aspect of the left thigh and with involvement of the inguinal lymph nodes. Six months later she married and became pregnant. During her pregnancy cutaneous nodules were noted. Sixteen months after the first diagnosis was made a cesarean section was done and a living baby delivered. The placenta was large and dark brown, and melanotic tumor cells were found in the blood vessels of a chorionic villus. The mother expired three months later as a consequence of metastatic spread. The baby seemed well for the first five months but at the age of eight months she was admitted with an enlarged liver and died at the age of ten and a quarter months. Autopsy showed widespread melanoma deposits in the liver, lungs and subcutaneous tissues. Histologic examination disclosed a tumor pattern essentially similar to that observed in the mother.

One of the clinical manifestations of extensive metastatic melanoma is the development of melanosis and melanuria, and even on occasion the presence of melanin in the mother's milk. Ehrlich's reagent[20] has been used to demonstrate melanogens and gives a characteristic red color reaction.

THERAPY

There is general agreement that the present day treatment of malignant melanoma is primarily surgical. An excisional biopsy is the first step in obtaining an accurate diagnosis. Once the diagnosis is soundly established, the type of surgical management is determined by such factors as the anatomic site of the tumor and the presence or absence of lymphatic or blood stream metastasis.

For ease of discussion the cases are divided into four groups.

GROUP I. CASES WITH CLINICALLY EVIDENT BLOOD-STREAM METASTASIS

Patients presenting the clinical picture of distant blood borne metastases offer no practical *possibility* of *effecting* a cure, and hence nothing but palliative measures should be considered. Of course allowance must be made for that patient, for example, who appears with a solitary brain metastasis after a long period of freedom from disease following previous surgical treatment of a skin melanoma.[24] Surgical removal of such a nodule offering—as it does in many instances—several years of happy life to the patient, completely justifies the procedure.

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disabling and mutilating nature of such surgery, however, has made the problem of securing consent extremely difficult.

GROUP IV. CASES WITH INOPERABLE REGIONAL LYMPH NODE METASTASIS

Patients with densely matted and partially or completely fixed lymph nodes should not be subjected to operation. The possibility of complete removal is practically nil and complications such as fungation of the accelerated tumor and poor wound healing are formidable. In such cases radiation therapy is sometimes a useful palliative measure.

Other types of treatment have been tried. Attempts to control the melanoma by massive doses of hormones have resulted in nothing but failure. Roentgen ray and radium therapy offer little more, though still used to some extent as an adjunct to surgery and for palliation. Marcus and Rotblat [3] recently reported their work on a new form of radiation therapy with radioactive isotopes. Uptake studies were carried out on the isotopes P^{32}, I^{131} and Cu^{64}. The radioactive iodine and copper were not taken up by the melanomas in any significantly selective fashion. The P^{32} uptake was noted as 4 to 10 higher than the uptake of normal skin; hence a patient with widespread metastatic melanoma was given therapeutic doses of this isotope. Although there appeared to be some inhibition of the growth rate of the metastatic tumors, the clinical course of the disease was not materially altered. The value of this form of experimental therapy will have to await further study.

PROGNOSIS

Any attempt to evaluate the prognosis for patients with malignant melanoma demands that due consideration be given to the variable biologic behavior of the tumor, the method and adequacy of treatment, and to a host of other factors. The failure to do this results in misleadingly gloomy or euphoric statistics.

Several authors classify the disease by stages [1,40] using the following categorization as a basis:

Stage I. Negative nodes.
Stage II. Clinically negative nodes which are microscopically involved.
Stage III. Clinically positive regional nodes without distant metastases being evident.
Stage IV. Distant metastases.

Where such staging is adopted the five year survival rate for Stage I patients is in the neighborhood of 30 to 40 per cent—when the treatment is early and adequate. Prognosis becomes progressively worse with extension of the lesion. Patients with palpably involved regional nodes (Stage III) have a five year survival rate of approximately 5 per cent.[8] In patients with distant metastases (Stage IV) the five year survival is virtually zero.

With regard to prognosis as it relates to the site of the primary, it appears that lesions on the head usually have the best survival value.[1] Many statistical summaries, however, are not compiled to show this fact and consider the head and neck together. The more auspicious prognosis for melanomas located on

the head is largely due to the fairly well delineated lymphatic drainage with readily accessible nodes But when the lesion is on the neck the scene changes and for this reason it would seem better to separate the two sites when evaluating prognosis Melanomas of the back and chest have a poor prognosis[1] for there is extreme likelihood of deep lymphatic drainage

Five year survival rates are not accurate measures of freedom from the tumor process for the melanoma seems especially prone to recur after this period [10 46] The practice followed by some investigators[10 40] of reporting the actual status of the patients at the time of writing—in addition to the conventional five year survival values—is to be commended

The malignant melanoma is not 100 per cent predictable, and the whole question of prognosis must as yet end on an uncertain note All that can be said in the light of our present knowledge is Approach the Stage I and II patient with a hopeful but guarded outlook the Stage III and IV victim with extreme pessimism

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disabling and mutilating nature of such surgery, however, has made the problem of securing consent extremely difficult.

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Chapter

3

PATHOLOGY OF SYSTEMIC LUPUS ERYTHEMATOSUS*

By Paul Klemperer, M.D.

In preparing a review of recent advances in our knowledge of systemic lupus erythematosus I found myself confronted with a dilemma. What period should be chosen as the starting point for my survey of the progress which has been achieved in the understanding of the disease? Should I limit myself to a discussion of the most recent investigations only and advise the reader to consult comprehensive articles such as that of Montgomery[1] or Baehr and Jarcho for the state of information as presented in 1939 or 1930? It certainly would have relieved me of laborious studies of the old literature. It also would have exculpated me of reproaches of having omitted pertinent articles on the subject since it is hardly possible to cover the vast literature which has been accumulated in the past century when the term lupus erythematosus has been introduced by Cazenave.[3] But I would have deprived myself of a most pleasant task of historical recollections which is so peculiarly attractive to the advancing years of the student of medicine. In this period of his life he enjoys delving into the past to try to connect the discoveries of past generations with the achievements of the present. He finds encouragement in the recognition that each generation has been building for the future; he tries to understand the investigative principles which have guided the investigators of the past and wishes to recognize why they have failed to grasp facts which have been revealed by recent research only. Such an approach will protect him from becoming a *laudator temporis acti* and might stimulate the interest of younger students of medicine.

HISTORICAL

The history of systemic lupus erythematosus begins with Kaposi's[4] description of the acute forms of the malady. However Kaposi believed in a fundamental identity of the chronic and acute disease and even today the relationship between the two forms has not been fully clarified. For this reason a short review of the history of lupus erythematosus might be justified. Willan,[5] the founder of a nosologic classification of cutaneous diseases on morphologic principles was the first to give a clear description of the various appearances of lupus as it destroys the skin of the face. His pupil Bateman,[6] who completed his master's work, seems to have been the first who noted the peculiarities of lupus erythematosus and it is interesting that he did not confuse it with Willan's lupus. However he classified it as simple ichthyosis limited to the face, having apparently been impressed by the striking scaling of the epidermal surface. Plate XVIII of his

* Supported by a grant from the U. S. Public Health Service.

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This ready acceptance of a bacterial etiology in particular that of the tubercle bacillus has delayed progress in our understanding of the nature of lupus erythematosus especially that of the systemic type Boeck[155] in 1880 described a series of cases which he believed to be identical with the acute disseminated lupus erythematosus of Kaposi and could prove their tuberculous nature Although subsequently it was recognized that Boeck s lupus erythematosus was fundamentally different from Kaposi s acute lupus erythematosus and was generally identified with tuberculides (Boeck[156]) the tubercle bacillus continued to play a major role in considerations of the etiology of the disease particularly of the acute variety Pathologic anatomic observations were quoted mainly in reference to the question whether tuberculous lesions were found in the body or not Without critique the presence of active or silent tuberculous infection was accepted as significant Of course there were students of the disease who did not accept a tuberculous etiology and who found support for their skepticism in necropsy reports in which the absence of tuberculosis was established One is surprised at the narrow concentration of interest and wonders why the great pathologic anatomists of that period did not make any attempt to clarify the obvious puzzle of acute lupus erythematosus In other words one questions how it was possible that during the period from 1872–1924 there is hardly one necropsy report on record which foreshadows the anatomic observations of recent times I believe there are several reasons which account for the peculiar sterility foremost among them the lack of clear pathogenetic thinking which is characteristic for medicine of the second part of the nineteenth century and particularly for dermatology It had been fully understood by Rayer early in the century that peculiar and characteristic cutaneous symptoms might be associated with general diseases ' the effects of which are occasionally felt with far greater violence by other systems than the skin This general point of view admirably expresses a concept which applied to systemic lupus erythematosus only gradually developed over a period of nearly eighty years

The influence of Hebra and his school who had established the principle of idiopathic skin diseases was of course of far reaching significance for the sound development of dermatology based on exact observation Yet this accuracy of observation of detail and even Hebra s attempt to classify cutaneous lesions according to the principles of Rokitansky s pathologic anatomy could not lead to an understanding of the nature of cutaneous maladies The general tendency of medicine was to establish exact descriptive criteria of disease only and it refrained from every attempt of interpretation of morbid phenomena It was this attitude of mind which prevented Kaposi the discoverer of acute lupus erythematosus from recognizing its nature as a systemic malady Moreover emphasis upon morphologic detail led to overspecialization and dermatology became isolated The interest in cutaneous diseases of the general pathologist waned and skin pathology was separated from the body of general pathology The history of acute lupus erythematosus well illustrates the dangers inherent in the tendency of specialization in medicine The lack of interest on the part of general pathologists in diseases of the skin most probably accounts for the dearth of significant observations at the autopsy table and this in turn contributed greatly to the confinement of the nosologic definition of acute lupus erythematosus to a most detailed but nevertheless superficial descriptive characterization as a skin malady Histologic investigations were limited to examination of the cutaneous lesions

"delineations" gives the first reproduction of the cutaneous lesion (1817). Rayer,[7] who apparently was also familiar with the gross morphological appearance, referred to the malady as *flux sébacée* (1837) and opposed Bateman's classification which was subsequently dropped by Thompson,[8] who edited the eighth edition of Willan-Bateman's book. The term *flux sébacée* pointed to a pathogenetic interpretation of the lesion by Rayer, as originating in an affliction of the sebaceous glands (1826).[9] This is of interest because ten years later Hebra[10] applied the same interpretation in his description of the disease and used the term "congestive seborrhoea." In the meantime, however, the varying characteristics of the disease became evident to Biett. In the first edition of Cazenave's and Schedel's[11] practical treatise on skin diseases compiled according to the clinical lectures of Biett, the authors give a description which well conforms with that of lupus erythematosus. However, it is included among the destructive skin lesions to which the French School applied the term *Dartre rongeante*. Because of its less destructive character Cazenave and Schedel classified it as *Dartre rongeante qui détruit en surface*. In spite of this separation the malady is included with the destructive skin lesions which had already been classified as Lupus by Willan. Biett, however, must have been aware of similar skin lesions without destructive character because Cazenave and Schedel mention in their third edition an entity called erythema centrifuge and state categorically in the fourth edition (1838) that both manifestations refer to one and the same malady. Consequently Cazenave[3] simplified the terminology and used the term *lupus erythematosus* in his classical contribution in 1851.

The identification of the disease as an entity found general approval and the term was soon universally accepted. Even Hebra, who had given an excellent description of the same disease under the term congestive seborrhoea, used the new name in his notes accompanying the plates in his Atlas der Hautkrankheiten[1] which reproduce in an admirable veracity the morphologic characteristics of the cutaneous manifestations. One is amazed that he conceded so readily his term, but more so, that he did not defend his pathogenetic concept which he obviously expressed by the choice of the term congestive seborrhoea. What was the reason for Hebra's acquiescence? Was it mainly his conciliatory attitude toward nomenclature which he demonstrated in the preface to his Atlas with the words "never to use new names for old maladies"? Was it in deference to the tradition of the famous French School or was it, as Besnier and Doyon[13] later intimated, the realization that the term had not been created by him but by Rayer? One can only guess at the reasons but the fact that the term lupus erythematosus was so quickly and universally accepted tends to show that medical thinking of that period was still satisfied with defining morbid entities by descriptive characterization (Merkmals definition). As Michelson[14] pointed out, the term lupus had gradually come to be used for facial lesions which spread centrifugally and might heal centrally either with scar or atrophy. The addition of the adjective erythematosus seemed to be adequate to separate the entity from the more common forms of the classical Willan's lupus. Pathogenetic inquiry had fallen into disrepute because of the senseless speculation of the preceding period of Naturphilosophie and the time of bacteriology had not yet arrived. But when the tuberculous etiology of lupus vulgaris was established it was obviously also accepted for lupus erythematosus.

process within the dermis, the nature of the proliferating cells, their relation to blood vessels and to the changes of the collagen and elastic fibers. However one can only agree with Jadassohn's[15] critical remarks that the histologic findings do not permit any conclusion as to the nature of the pathologic process. While this conclusion referred mainly to the discoid forms of lupus erythematosus it applied even more to Kaposi's acute lupus erythematosus because detailed histologic observations of the viscera were entirely lacking and those of the skin very scarce and apparently similar to those of the chronic cases. Thus the inadequacy and sterility *of structural investigations could not contribute to an elucidation of the* pathologic process. On the other hand attempts of clinicians to interpret the alterations of the skin organ as well as the striking manifestations of the systemic disease were limited to meaningless generalizations such as inundation of the body by toxins, general infection, predisposing and constitutional factors. Progress of a more rational inquiry into the fundamental nature of acute lupus erythematosus was rather slow. It began with the realization of dermatologists that the cutaneous manifestations are only part and parcel of a generalized systemic malady *(Jarisch) and with the awakening of interest of internists in the disease.* Osler[3] no doubt was one of the first who recognized the relationship of skin lesions (erythema) to associated visceral manifestations, although he was not solely concerned with lupus erythematosus.

When acute lupus erythematosus began to be viewed from the broader aspect of internal medicine, the protean clinical picture began to emerge with greater clarity and pertinent laboratory findings such as leukopenia and thrombocytopenia were added to the diagnostic criteria (Goeckerman,[4] Keefer and Felty). Yet acute lupus erythematosus was still defined by clinical characteristics only and the time had not yet arrived for a rational inquiry into pathogenesis. Consequently one finds in the reports of the first decades of the twentieth century still the hazy notions of toxicity, tuberculous or otherwise (Roberts,[6] Goeckerman,[4] Kraus and Bohac[27]) embellished by fancy ideas of hypersensitivity (Stokes[28]), ferment dyscrasia (Gennerich[9]) or even that of tubercle bacillemia without allergy (Ehrmann and Falkenstein,[30] Kren,[31] review by Gawalowski,[3] Robertson and Klander[33]). An impasse had been reached from which even the most accurate description of symptoms could not offer an escape. A new approach had to be found.

In 1924 Libman and Sacks[34] published their classical report on a hitherto unknown form of endocarditis. Although the title of the article does not indicate it there is no question that it contains the first significant contribution to the pathologic anatomy of systemic lupus erythematosus. Two of the cases showed skin lesions similar to those of acute lupus erythematosus and the description of the general symptomatology fits very well the diagnosis. While the authors originally believed to have presented a new nosologic entity, it was later conceded by Libman that the clinical course of the disease and especially the visceral manifestations of their patients were those of acute lupus erythematosus. It is significant for the diagnostic uncertainty of this relatively recent period that Libman and Sacks did not recognize the similarity of their cases with acute lupus erythematosus. More so, however, that other authors did not detect it either. In fact this failure caused considerable confusion in subsequent years because the clinical picture together with the unusual endocarditis was referred to as Libman-Sacks disease and differentiated from acute lupus erythematosus.

and confined themselves to a conventional description of the conspicuous dermal changes with an attempt to classify them according to the static principles of general pathology prevailing in this period

In their early histologic reports of the skin alterations in lupus erythematosus Neumann[16] and Geddings[17] refer to a chronic inflammatory process without characteristic features except its site of origin around sebaceous glands This observation was challenged by subsequent investigators Later studies (see Jadassohn[18]) carried out with improved technique led to a more precise description of the histologic picture and consequently established fairly accurate diagnostic criteria However none of these studies contributed to a clearer understanding of the fundamental process responsible for the tissue alteration Summarizing the reports of dermatopathologists of the preceding period Jadassohn[18] aptly remarks that none of these studies has clarified the pathogenesis of the disease, and this judgment continued to apply to the investigations of dermatopathologists of a later even very recent, period The frustrating inadequacy of static morphologic research for the explanation of disease focussed the main interest upon etiology The impact of bacteriology upon medicine and general pathology in the second part of the nineteenth century extended to dermatology and obviously to that puzzling malady, lupus erythematosus The fashion of exclusively etiologic thinking of this period which Rosenbach[19] critically characterized as the era of only bacteriology (*Nichts als Bakteriologie*) is well summarized in Jadassohn s[18] Chapter IV on occurrence and etiology After an exhaustive survey of the entire literature and a careful analysis of the prevailing hypotheses he comes to the conclusion that he cannot recognize a constant connection of lupus erythematosus with tuberculosis or any other bacterial cause and this conclusion applies both to the chronic and the acute forms of the disease Yet in spite of this refutation by a recognized leader in the field of dermatology the tuberculous origin of lupus erythematosus continued in the forefront of all discussions regarding the nature of the disease As late as 1934 Keil[20] again in a critical analysis showed the fallacy of attributing etiologic significance to tuberculosis While today the majority of investigators agree with this point of view there are still occasional publications which refer to a possible role of the tubercle bacillus in the causation of the disease (Ronzani[21])

PRESENT DAY CONCEPTS

Fortunately however it is generally recognized today that the understanding of the nature of a disease does not depend exclusively upon the discovery of its cause The search for mechanisms to explain the basic alterations of form and function of the body characteristic for a disease has become the principle of investigative thinking in modern medicine

It might be of interest to trace the evolution of this heuristic concept in the history of lupus erythematosus Rayer s and Hebra s original contention that the disease begins with an anomaly of the secretion of the sebaceous glands seemed first to be supported by the early histologic investigations of Neumann and Geddings This idea however became untenable when it was shown that cutaneous lesions occurred in skin areas free of sebaceous glands such as in the palm of the hands and on mucous membranes Subsequent dermatopathologic studies added many divergent details regarding the exact primary site of the pathologic

was interpreted as modified ground substance It was stressed that the visible alterations were the manifestations of a change in the colloidal state of the intercellular substances and the hypothesis was offered that physicochemical changes may occur without detectable structural alterations Such a possibility could account for those observations in which the pertinent lesions were minimal or even absent

From these observations the conclusion was reached that systemic lupus erythematosus was defined anatomically by a systemic alteration of the connective tissue especially of its extracellular components The conspicuous fibrinoid changes of the connective tissue were regarded according to the classical observations of Neumann[46] as degeneration of the collagenous fibers However it was recognized at that time and subsequently stressed that fibrinoid alteration of the collagenous tissue is incompletely understood This structural alteration reflects a disturbance of the physicochemical state of the intermediate substances of the connective tissue It does not necessarily denote a state of inferiority in a biologic sense of the connective tissue fibers and should not be designated as fibrinoid degeneration In fact, it has become evident in recent years that the substance referred to as fibrinoid deserves far more searching inquiry It cannot be accepted that it derives only from collagen fibers in many instances it is a deposition of different proteins between or upon the collagen fibers The sclerosing changes of the connective tissue deserve also far more scrutiny than they have received so far These are problems which lead into an inquiry into the chemical and physical constitution of the intermediate substances and the factors which control their plasticity under normal and abnormal conditions Such questions cannot be solved by morphologic investigation alone but they have been provoked by morphologic observations

Returning to the discussion of systemic lupus erythematosus it was recognized that fibrinoid or sclerosing connective tissue changes had been observed in rheumatic fever as well as in generalized scleroderma Thus it was apparent that obviously heterogeneous diseases had in common a systemic alteration of the extracellular components of the connective tissue But this common seat of anatomical alteration did not necessarily mean identity of pathogenesis This point of view was supported by the recognition of clear cut differences in the most characteristic organ lesions in these maladies Thus the conspicuous wire loop lesions and hyaline capillary thrombi of the glomeruli in systemic lupus erythematosus had never been observed in rheumatic fever while Aschoff bodies were never found in systemic lupus erythematosus Only in one case of generalized scleroderma (Pollack 1940), were glomerular changes observed and fibrinoid endocardial lesions identical with those of acute lupus erythematosus while no other case investigated at that time and subsequently presented a similar picture A recent re-examination of this isolated case showed the pathognomonic feature of hematoxylin bodies which were never observed in any other case of our material of generalized scleroderma In the light of these recent studies it can be concluded that the case reported by Pollack represents a coincidental combination of systemic lupus erythematosus and generalized scleroderma

It was repeatedly stressed by us that it would be erroneous to identify diseases with one another because they share the site of anatomical alterations By suggesting the term diffuse collagen diseases we[47] unwittingly became responsible for a deplorable usage of this term as a collective diagnosis for those puzzling

The confusion was clarified by the contributions of Belote and Ratner [35] Keil[36] and especially subsequent papers of Libman [37] The latter emphasized that cutaneous lesions need not be conspicuous and might even be absent in the syndrome and consequently frowned upon the diagnostic term acute lupus erythematosus However Hebra already had resigned himself to the use of Cazenave s term although he was aware that the name was not very well chosen After all terms do not matter if there is comprehension attached to them, which is clearly shown by the recent development of diagnostic criteria for systemic lupus erythematosus Libman and Sacks[34] certainly deserve credit for having broadened the descriptive characterization of systemic lupus erythematosus by discovering one of the pathologic anatomic lesions of the disease A perusal of their microscopic description reveals that they had also observed certain histologic changes such as periarterial follicular sclerosis of the spleen* and the presence of hematoxylin stained bodies in the endocardial vegetations which were destined to play an important role in subsequent pathogenetic interpretations The specificity of these hematoxylin stained bodies for the endocardial vegetations in acute lupus erythematosus was later established by Gross[39] (1932 and 1940) and their occurrence in other organs (lymph nodes and kidneys**) by Ginzler and Fox [40] subsequently by Klemperer Pollack and Baehr [4] (1941) and again by Klemperer Gueft Lee, Leuchtenberger and Pollister[43] (1950) While Libman and Sacks [34] and Gross [39] (1940) had concentrated their pathologic anatomic investigations upon the heart, Baehr Klemperer and Schifrin [41] investigating the other organs in systemic lupus erythematosus observed conspicuous vascular lesions which had hitherto escaped recognition Not fully satisfied with a mere descriptive characterization of the pathologic anatomy of acute lupus erythematosus they expressed the belief that the association of changes in the serosal coats endocardium blood vessels and synovial membranes pointed to the endothelial cells as the site of primary attack of a noxa in a broad sense Keil[45] also saw in vascular lesions the fundamental principle of the pathogenesis of systemic lupus erythematosus and formulated a vascular concept of the disease

In the light of subsequent investigations these pathogenetic generalizations have become untenable Upon observation of new and re examination of old cases Klemperer Pollack and Baehr[4] found that a significant number showed conspicuous and systemic alterations of the extracellular components of the connective tissue The most conspicuous feature of these changes was fibrinoid alteration of the collagen fibers and periarterial sclerosis of the spleen Since connective tissue is a constituent of all organs of the body its systemic implication explained the apparently heterogeneous involvement of numerous organs as it had been observed in previous investigations Morbid states of the skin the heart the vessels serous membranes and joints could all be reduced to the common denominator of an alteration of the connective tissue specifically of its extracellular components It was recognized that the homogeneous ground substance was also affected because the abnormal fibers were glued together and because the glomerular wire loop changes could not be regarded as fibrinoid alteration of collagen fibers but rather as a change of the basement membrane which

* Volk[3] in 1928 recorded an identical observation His case showed conspicuous vessel changes which he regarded as periarteritis nodosa

** Lunitschek and Kettner[4] apparently had seen similar lesions and referred to them as circumscribed loop necroses of toxic origin

be further advanced before we should approach the question of their pathogenesis

As far as acute lupus erythematosus is concerned pathologic anatomic reports subsequent to our publication in 1941 mostly confirmed our observations (Rakov and Taylor[50] Teilum[51] Foldes[5])

However, it became evident to me that the pathologic anatomic definition was inadequate particularly from a diagnostic point of view It had been recognized that not every case of the disease showed the pathognomonic cardiac renal or vascular lesions Moreover the differentiation from other diseases showing fibrinoid vascular alterations was equivocal (Mallory,[53] Rich[54] Bauer, Kulka and Giansiracusa[55]) The periarterial splenic sclerosis was observed though rarely, in other morbid conditions (Kaiser[56]) and not persistently found in systemic lupus erythematosus Clinical investigators (Coburn and Moore[57]) pointed to ' the striking disproportion between the extensiveness of the clinical manifestations and the paucity of histological lesions and suggested ' that the disease process was primarily one of altered physiology which could best be studied in vivo While such statements revealed a misconception of the role of structural investigations in the inquiry into the nature of disease they stimulated a search for additional criteria in the post mortem diagnosis of systemic lupus erythematosus

In 1941 we had called attention to the fibrous pericardial adhesions which often have a strikingly thick, gelatinous succulent appearance Low Logan and Rutherford[58] and Mook Weiss and Bromberg[59] referred to edematous fibrous tissue in the greatly thickened epicardium of their cases The development of reliable staining methods for mucoproteins led me to reinvestigate several hearts of new cases of lupus erythematosus and the presence of large amounts of metachromatic substance was noted within the widened septa of the myocardium and also within pericardial adhesions[60] Subsequent examinations by Dr Lee in our laboratories showed that the staining reaction was abolished by preceding treatment of the section with bull testis hyaluronidase A differential determination between hyaluronic acid and chondroitinsulfate was not accomplished because of the lack of potent streptococcal enzyme These observations which were also made in sections from retroperitoneal tissue supported the thesis that the intermediate substances of the connective tissue in their totality are implicated in systemic lupus erythematosus Yet they did not add a further pathognomonic feature because in rheumatic fever metachromasia is also found in and around Aschoff nodules and even nonspecific granulation tissue is rich in this material

In the meantime observations of 4 new cases drew our attention to the possible significance of the hematoxylin bodies originally described by Gross[39] and Ginzler and Fox[40] as a diagnostic criterion In 1950 we[43] reported that in 32 of 35 cases of acute lupus erythematosus these characteristic tissue lesions had been found Since this report has been presented 12 additional consecutive cases were examined and hematoxylin bodies were found in every one and not only in one site but in many organs * These investigations were carried out by Drs Gueft and Laufer and will be published shortly The conclusions drawn from our previous studies were fully confirmed and amplified by the study of these twelve new cases

* Two of these cases had been treated with cortisone one of them for a period of one and one half years Both cases did not differ from the untreated ones

maladies which we had tried to differentiate by rational pathogenetic investigation When we proposed the term we wanted only to call attention to the significance of systemic alterations of the connective tissue in certain maladies It was repeatedly pointed out by us that it was the aim of further investigations to disclose the basic factors responsible for aberrations of the structure of the collagenous tissue in order to define the diverse morbid entities which constitute the group collectively termed collagen diseases It was necessary to stress that the very changes of the intermediate substances of the connective tissue deserve far more searching inquiry than they had received so far

We had not been the first who had called attention to systemic connective tissue implication and who had recognized its significance for pathogenetic interpretation Klinge[48] in 1933 masterly described the diffuse involvement of the connective tissue in rheumatic fever and rheumatoid arthritis He called attention to the fibrinoid connective tissue changes and to the myxomatous swelling of the ground substance and recognized that these changes were of fundamental importance for the genesis of the rheumatic diseases But he did not recognize the complexity of these tissue alterations Instead of a patient analysis of the morphologic changes he singled out the fibrinoid tissue damage as the basis for his biologic anatomic synthesis which should explain the pathogenesis of rheumatic diseases According to him and those who followed him fibrinoid connective tissue changes were the manifestations of hypersensitivity Consequently all maladies characterized anatomically by such changes were supposed to be of an allergic background The general terms rheumatic, allergic or pararheumatic were applied to denote the pathogenetic identity of all these maladies Obviously acute lupus erythematosus would belong to this collective group and subsequent to our publication in 1941 many renowned investigators have advocated an allergic etiology for the disease because of fibrinoid connective tissue alteration For several reasons we refused to accept the thesis Firstly our clinical observations had not revealed any of the conventional symptoms and laboratory findings of hypersensitivity secondly we were aware that local fibrinoid connective tissue change was frequently observed in situations in which allergy could definitely be ruled out and well defined other causative factors be found such as enzyme action etc But beyond these reasons we felt that to accept the allergic hypothesis meant only to explain an obscure structural alteration by an equally obscure pathogenetic mechanism We believed that an inordinate preoccupation with immediate cause would shut the door to an analysis of the nature of the connective tissue damage in all these puzzling diseases

Obviously investigations of tissue changes in disease cannot remain at the level of mere description They must be finally directed at correlating structural alterations with aberration of function But investigations in anatomic pathology aim primarily at a comprehension of the physical and chemical factors responsible for visible changes of structure Such investigations rest upon an exact knowledge of the normal An analysis of the alterations of the intermediate substances in disease had to be founded upon adequate knowledge of their normal states When we surveyed the existing state of information regarding the intermediate substances of the connective tissue we became aware of a serious inadequacy of our knowledge of facts Since these problems have been presented by me only recently [49] I shall not enter into a further discussion We believed that inquiry into the constitution and biology of the intermediate substances had to

PLATE 1

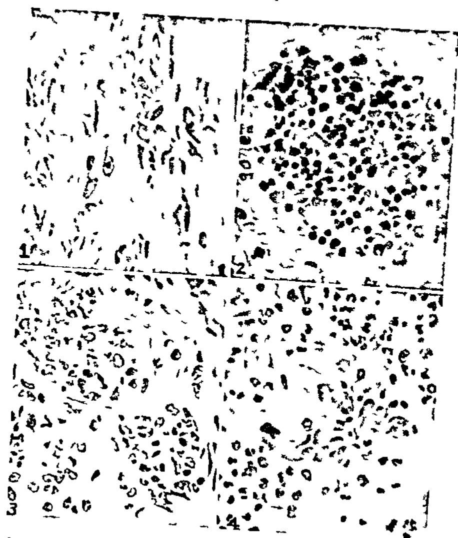


Fig 1 — Section of grossly normal mitral valve showing numerous small haped hematoxylin stained bodies without surrounding cytoplasm (× 441)

Fig 2 — Area in biopsy lymph node showing altered hematoxylin stained bodies with homogenization and purple staining of lymphocytic nuclei (× 441)

Fig 3 — Glomerulus showing numerous purple stained hematoxylin stained bodies (× 441)

Fig 4 — Pleura showing small aggregation of hematoxylin stained bodies and one histiocyte (arrow) with nuclear homogenization and purple staining (× 441)

(*Klemperer Gueft Lee Leiter chtenberg and Pollister ... Pathology*)



FIG 16 —*Coarse verrucous endocarditis* Vegetation with large aggregate of hematoxylin stained bodies (× 120) Klemperer *et al* Arch Path)

FIG 17 —Large aggregates of hematoxylin stained bodies in a sinus of a necrotic lymph node (× 120) (Klemperer *et al* Arch Path)

Summarizing the hematoxylin stained bodies occur in larger aggregates mainly in the endocardial vegetations (Fig 16) and in lymph nodes (Fig 17) Smaller collections and single free bodies with identical tinctorial qualities are found within the renal glomeruli, vascular wall of many organs and widely distributed in the connective tissue at various sites (Plate I) The bodies were seen in a high percentage of the cases in our first series and never missed in the second group of twelve consecutive cases While in the first series hematoxylin stained bodies were found in two or more organs in 24 of the 35 cases they were identified in most of the organs in each case of the second series

The study of variegated control material including cases which have been regarded as related for clinical or pathologic anatomic reasons did not reveal similar lesions The ovary proved to be an excellent organ for such control studies because within the wall of blood vessels in this organ the characteristic bodies could be easily found after short examination in systemic lupus erythematosus while intensive and prolonged search did not reveal them in other maladies From these observations it can now be concluded that the hematoxylin stained bodies are the pathognomonic criterion for the post mortem diagnosis of systemic lupus erythematosus

About the same time these autopsy studies were made, Hargraves Richmond and Morton[61] independently made their important discovery of the lupus erythematosus cell in the bone marrow of patients suffering from systemic lupus erythematosus Their findings have been confirmed by an ever increasing number of investigators and the specificity of the lupus erythematosus cell found in the bone marrow as well as in the circulating blood (Sundberg and Lick[6]) has been fully established Thus a valid criterion has been found for the diagnosis of systemic lupus erythematosus in the living *

With these contributions accomplished by the methods of morphology the efforts to reach an unequivocal definition of systemic lupus erythematosus by descriptive characterization have reached their conclusion The time has now arrived to enter into a rational inquiry into the pathogenesis of the disease and to explore the possible mechanism by which the structural alterations are provoked and to explain the striking and unique clinical symptoms of the disease

With the observation of generalized alterations of the intermediate substances of the connective tissue in systemic lupus it was recognized that their pathogenetic significance should be investigated It was evident that such investigations would not directly attack the specific problem of systemic lupus erythematosus because explorations of so wide a scope required the correlation of facts established by fundamental research as well as by careful observations of medical investigators Obviously the perplexing question as to the nature of the intermediate substances and their implication in disease could not be limited to an inquiry into the tissue changes in systemic lupus erythematosus But it could be anticipated that a greater insight into the biology of the connective tissue would enlighten our understanding of systemic lupus erythematosus and that conversely persistent analysis of the morbid features of this disease might provide some clue for the solution of the more universal problem While in our laboratories investigations were carried out on the influence of hormones on the deposition of ground substance in the chick comb and in the orbit of the guinea pig and while the ever

* Cortisone and ACTH treatment influenced the number of lupus erythematosus cells but did not cause their complete disappearance

DNA depolymerization apparent from our investigations of tissue lesions and bone marrow aspirates in systemic lupus erythematosus Studies to clarify this question are at present carried out by Drs Lee and Kurnick

It has previously been stated that the exploration of the nature of the hema toxylin bodies and of the mechanism of their formation seemed to bear no relation to the problem of the striking alterations of the intermediate substances of the connective tissue in systemic lupus erythematosus Recent investigations of the glomerular and vascular lesions of a number of such cases seem to establish an interesting relationship between these apparently heterogeneous morbid tissue manifestations In 1935 Baehr Klemperer and Schifrin[44] called attention to hyaline thrombi within glomerular capillaries in association with the so called wire loops (Fig 1A of the article) In 1941 we again recorded this observation (Fig 6B of this article) and stated that the hyaline thrombi stained identically with the substance of the glomerular wire loops Upon examination of new mater ial it was noted that some of the hyaline thrombi stained with hematoxylin eosin showed smudged particles faintly staining with hematoxylin Feulgen reaction of these particles was positive Since there were transitions found from typical hematoxylin bodies to the eosinophilic hyaline thrombi it was believed that the latter were derived from the former The hematoxylin bodies are nucleo proteins containing depolymerized DNA From our observations it is suggested that progressive depolymerization of the DNA may lead to a complete decomposi tion of the DNA and that the hyaline thrombi may consist of the residue of the broken down nucleoprotein The protein moiety is formed by histone and a non histone protein containing tyrosine and tryptophane (Mirsky and Pollister[68]) The hyaline thrombi are digested by trypsin they are Millon positive stain deeply with fast green and brilliantly with orange G of Mallory s trichrome mix ture Recently White[69] reported that purified histone has a great affinity for orange G The protein nature of the hyaline thrombi as evidenced by the histo chemical tests supports the deduction that they are derived from a progressive breakdown of the more complex nucleoprotein They are P A S positive even after trypsin digestion and might therefore contain additional carbohydrates Hyaline thrombi sometimes with a faint hematoxylin tint are also seen outside the glomeruli within the intertubular capillaries and hematoxylin bodies of vary ing intensity of hematoxylin stain are frequently seen in blood vessels

From these observations and the findings of lupus erythematosus cells in blood smears (Sundberg and Lick[6]) it might be assumed that free nucleoproteins in various stages of decomposition are circulating within the blood of patients with systemic lupus erythematosus This is protein which is foreign to the normal blood proteins It is now interesting to refer to our previous statments that the hyaline thrombi stain identical with the glomerular wire loops and that the latter are digested by trypsin This observation was fully confirmed by recent examina tions The tinctorial behavior has led us (1941) to consider the wire loops as consisting of fibrinoid material The fibrinoid material in larger vessels is fre quently associated with hematoxylin stained bodies with varying intensities of the hematoxylin tint

From these observations it might be concluded that the fibrinoid material in glomerular capillaries and in the vessel wall is derived from a progressive break down of nucleoproteins and represents histone and other protein residues which impregnate the vessel wall Since the process of DNA depolymerization is

increasing literature on the complex problem of the connective tissue was carefully integrated the recognition of the diagnostic significance of the hematoxylin bodies in systemic lupus erythematosus arrested our attention It occurred to us that this structural alteration should be explored for its pathogenetic significance although it seemed to bear no relationship to the intermediate substances of the connective tissue By appropriate cytochemical studies it was found that the hematoxylin bodies contained depolymerized desoxyribose nucleic acid (DNA) and it was concluded that one of the pathogenetic factors of systemic lupus erythematosus was a disturbance of DNA metabolism At the same time it was recognized that the characteristic inclusions of Hargraves lupus erythematosus cells bore a close resemblance to the small hematoxylin stained bodies in the tissue (Fig 18) Drs Lee Michael and Vural[63] examined therefore bone marrow



Fig 18 —Section of pleura (H E) showing a polymorphonuclear leucocyte with a large spherical inclusion The similarity to Hargraves L E cell is striking

aspirates of lupus patients with the same photometric methods which had previously been applied to the study of the hematoxylin stained bodies in tissues They found a similar increased ratio between optical density of Feulgen stained and methyl green stained inclusions compared with the respective optical densities of normal cells It was concluded that the inclusions of Hargraves lupus erythematosus cells are optically and chemically identical with the hematoxylin stained bodies These disclosures are of interest because Haserick and Bortz[64] and Hargraves[65] have shown that blood plasma of lupus patients contains a factor which is capable of inducing the production of lupus erythematosus cells from normal bone marrow cells Haserick[66] succeeded in localizing this factor in the gamma globulins by electrophoretic protein separation This factor is antigenic and rabbits injected with gamma globulins of lupus patients develop antibodies capable of preventing the lupus erythematosus plasma phenomenon (Haserick and Lewis[67]) It is suggestive that this plasma factor might be related to the

DNA depolymerization apparent from our investigations of tissue lesions and bone marrow aspirates in systemic lupus erythematosus. Studies to clarify this question are at present carried out by Drs. Lee and Kurnick.

It has previously been stated that the exploration of the nature of the hematoxylin bodies and of the mechanism of their formation seemed to bear no relation to the problem of the striking alterations of the intermediate substances of the connective tissue in systemic lupus erythematosus. Recent investigations of the glomerular and vascular lesions of a number of such cases seem to establish an interesting relationship between these apparently heterogeneous morbid tissue manifestations. In 1935 Baehr, Klemperer and Schifrin[44] called attention to hyaline thrombi within glomerular capillaries in association with the so called wire loops (Fig. 1A of the article). In 1941 we again recorded this observation (Fig. 6B of this article) and stated that the hyaline thrombi stained identically with the substance of the glomerular wire loops. Upon examination of new material it was noted that some of the hyaline thrombi stained with hematoxylin eosin showed smudged particles faintly staining with hematoxylin. Feulgen reaction of these particles was positive. Since there were transitions found from typical hematoxylin bodies to the eosinophilic hyaline thrombi it was believed that the latter were derived from the former. The hematoxylin bodies are nucleoproteins containing depolymerized DNA. From our observations it is suggested that progressive depolymerization of the DNA may lead to a complete decomposition of the DNA and that the hyaline thrombi may consist of the residue of the broken down nucleoprotein. The protein moiety is formed by histone and a non histone protein containing tyrosine and tryptophane (Mirsky and Pollister[65]). The hyaline thrombi are digested by trypsin, they are Millon positive, stain deeply with fast green and brilliantly with orange G of Mallory's trichrome mixture. Recently White[69] reported that purified histone has a great affinity for orange G. The protein nature of the hyaline thrombi as evidenced by the histochemical tests supports the deduction that they are derived from a progressive breakdown of the more complex nucleoprotein. They are P.A.S. positive even after trypsin digestion and might therefore contain additional carbohydrates. Hyaline thrombi sometimes with a faint hematoxylin tint are also seen outside the glomeruli within the intertubular capillaries and hematoxylin bodies of varying intensity of hematoxylin stain are frequently seen in blood vessels.

From these observations and the findings of lupus erythematosus cells in blood smears (Sundberg and Lick[8]) it might be assumed that free nucleoproteins in various stages of decomposition are circulating within the blood of patients with systemic lupus erythematosus. This is protein which is foreign to the normal blood proteins. It is now interesting to refer to our previous statments that the hyaline thrombi stain identical with the glomerular wire loops and that the latter are digested by trypsin. This observation was fully confirmed by recent examinations. The tinctorial behavior has led us (1941) to consider the wire loops as consisting of fibrinoid material. The fibrinoid material in larger vessels is frequently associated with hematoxylin stained bodies with varying intensities of the hematoxylin tint.

From these observations it might be concluded that the fibrinoid material in glomerular capillaries and in the vessel wall is derived from a progressive break down of nucleoproteins and represents histone and other protein residues which impregnate the vessel wall. Since the process of DNA depolymerization is

specific for systemic lupus erythematosus, it is justified to believe that the end product found impregnating the vessel wall is also characteristic In other words, it is suggestive that the fibrinoid in lupus specifically consists of the break down products of nucleoproteins, while fibrinoid occurring in other diseases is a different protein Fibrinoid as observed in various pathologic conditions is neither structurally nor chemically well defined and similarities of staining reaction with the conventional histologic techniques do not denote identity It is obvious but must be stressed that the tentative conclusions as to the nature of fibrinoid in systemic lupus erythematosus do not rest solely upon one staining reaction but are inferred from the results of *systematic histochemical inquiry The histologic analysis* suggests that the fibrinoid material within glomerular capillaries as well as in the wall of arteries is derived from local breakdown of nucleoproteins It can not yet be stated whether this decomposition is initiated by a factor acting upon the DNA or upon the protein moiety of the nucleoproteins The question, whether extravascular deposits of fibrinoid are due to a similar local mechanism or due to the precipation within the connective tissue ground substance of circulating free products of the original nucleoproteins has not yet been decided Not infrequently, small foci of fibrinoid deposits within the myocardium are associated with the presence of many free hematoxylin bodies obviously derived from accompanying inflammatory cells On the other hand I have observed extensive deposits of fibrinoid material on the endocardial surface without much evidence of local cell accumulation and nuclear decomposition This question must await clarification by further histologic analysis such studies are at present carried out by Drs Gueft and Laufer

From the review of the pertinent investigations of systemic lupus erythematosus it seems justified to conclude that the diagnostic criteria of the malady are today fully established both in the living patient as well as post mortem Do the available data permit a conclusion as to pathogenesis? It seems evident that the period preceding the recognition of the characteristic morphological lesions did not contribute any satisfactory clue as to cause or mechanisms of the morbid state It is of historical interest only that Gruetz[70] speculated about a mesenchymal inferiority as the underlying pathogenetic principle because in his opinion inflammation played only a minor role in the skin lesions However Denzer and Blumenthal[71] *commenting on a case which showed classical autopsy findings suggested* that mesenchymal structures in general may be affected by the unknown underlying cause This mesenchymal implication might compromise the organism s capacity for the production of antibodies and thus explain the susceptibility of the patient with lupus erythematosus disseminatus to intercurrent infection A similar idea is expressed by Long and Aegerter [7] who believe that all the derivatives *of the primitive mesenchyme the mesenchymal defense unit not* only the fibrous connective tissue are affected in systemic lupus erythematosus Long and Aegerter take it for granted that the fibroblasts produce collagen and that any lesion of collagen structural or functional therefore denotes a morbid state of the fibroblasts I have repeatedly stressed that the alteration of the intermediate substances of the connective tissue (for brevity s sake referred to as collagen) deserves more searching investigation The principles of cellular pathology cannot be applied to diseases characterized by alterations of the intermediate substances until it has been demonstrated beyond any *doubt that they are products of the* fibroblasts only

No doubt hypersensitivity is today the most popular explanation of the patho

genesis of systemic lupus erythematosus This hypothesis rests mainly upon the observations of fibrinoid connective tissue lesions in post mortem material although the elevation of the gamma globulins (Coburn and Moore,[57] Walker Sheldon and Benditt[73]) and nowadays the beneficial effect of cortisone and ACTH on the course of the disease are quoted in support (Studer[74]) Teilum[75] regards the periarterial splenic fibrosis and the glomerular wire loop lesions as expressive of an allergic hyperglobulinosis I do not want to repeat my reasons for not accepting the thesis that fibrinoid alteration of connective tissue in human disease is pathognomonic for hypersensitivity As long as fibrinoid alterations were the essential morphologic findings in systemic lupus erythematosus, their pathogenetic interpretation as allergic was permissible Proponents of the hypothesis could justly maintain that no better alternative explanation was suggested by the opponents Today however, the adherents of the hypersensitivity theory are confronted with new facts which contradict their belief The lupus erythematosus cells have not been found in other conditions than systemic lupus erythematosus and the same holds for the hematoxylin bodies in tissues Special search was made for these criteria in diseases with allergic background such as polyarteritis nodosa acute and chronic glomerulonephritis and other conditions The findings of these criteria in systemic lupus erythematosus differentiate this disease from those which have generally been accepted as 'allergic' diseases Moreover recent observations have shown that the specific breakdown of nucleoproteins in systemic lupus erythematosus results in the deposition of fibrinoid material in glomerular capillaries and arterial walls It is obvious that fibrinoid in other morbid conditions which are not distinguished by the same metabolic alteration must be of a different nature These observations led me to the conclusion that the hypersensitivity hypothesis does not apply to systemic lupus erythematosus

While no complete theory can yet be offered to explain the pathogenesis of systemic lupus erythematosus the recent disclosures of morphological investigations have at least opened a new avenue for rational inquiry into the nature of the morbid process Haserick s discovery of a factor in the blood plasma responsible for the formation of lupus erythematosus cells in conjunction with the demonstration of DNA depolymerization in systemic lupus erythematosus suggests a search for the mechanism of action of this factor According to recent investigations[76] Haserick s L E factor seems to function as an inactivator of a desoxyribonuclease inhibitor normally present within polymorphonuclear leucocytes[77] The L E factor penetrates the cell membrane reacts with the inhibitor and thus the normally present desoxyribonuclease is free to attack the chromatin Whether the factor actually destroys the inhibitor or merely unites with it has not yet been worked out The demonstration of progressive breakdown of nucleoproteins in tissues makes a search for the final products such as histones and nucleotides in the plasma and urine of the patient advisable Such studies are now being carried out by Lee and Fresco in our laboratories

Summarizing the recent advances which have been made in the understanding of systemic lupus erythematosus it can be stated without hesitation that the progress is mainly due to morphologic investigations * No doubt the clinical

* In a recent presentation (*Transactions of the New York Academy of Sciences* 195 Series II 14 235 S E Moolten and Ellen Clark report that they have twice isolated a virus from a patient with systemic lupus erythematosus which was found capable of reproducing the L E cell phenomenon in vitro A human volunteer (the patient s husband) injected repeatedly with formalin inactivated virus developed a rising titer of neutralizing antibody This serum inhibited the L E cell phenomenon when mixed with the patient s serum or virus

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picture has been more clearly defined and some new chemical laboratory data have been revealed by electrophoretic protein partition and by determination of the hexosamines However the *significance of these observations for the under*standing of the nature of the morbid process has not yet been clarified On the other hand the discovery of the characteristic lupus erythematosus cell in bone marrow aspirates has secured the diagnosis in the living while the recognition of the hematoxylin stained bodies as a *constant element of the histologic findings* in tissues has established a criterion for the post mortem diagnosis These criteria have removed the uncertainty which until recently was attached to the diagnosis and has been responsible for the riddle of the malady These results alone would already have been of great importance However they have been only the starting point for further investigations into the mechanisms which are responsible for these structural alterations The discovery of an antigenic factor residing within the gamma globulins and the recognition of profound changes in nucleoprotein *metabolism* point the way *to further investigations of the blood proteins and their* relation to changes of the intermediate substances of the connective tissue The disclosures which are the result of systematic research of systemic lupus erythematosus may thus become of more general importance

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Chapter

4

THE DIAGNOSIS OF FUNGUS INFECTIONS WITH PARTICULAR REFERENCE TO STAINING METHODS

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Interest in medical mycology is reawakening The recent literature shows a sudden efflorescence of case reports of systemic fungus infections which were previously considered rarities This is not due to a sharply increased incidence but rather to an increased awareness of these formerly obscure conditions The mycoses have not become more common, information about them has

Bacteriologists and pathologists living in an age of specialization, nowadays are expected to be able to recognize and identify fungus infections Their interest in this field is therefore, entirely practical The recent publication of several good books on medical mycology has been of great help [1-3] As further evidence of the renewed interest in mycology one may note the ever increasing number of experimental studies undertaken by those with no special training in this field The recent recognition of the high incidence of mycotic pulmonary disease in certain endemic areas of the U S has compelled the attention of clinicians to this problem [4] In Southern California for instance primary pulmonary coccidioidomycosis is exceedingly common It mimicks other acute respiratory diseases closely Pulmonary histoplasmosis is another disease endemic in the Mississippi river valley and the plains states which complicates the problem of diagnosing lung disease A majority of individuals living in this area contract the disease (usually asymptomatically) at some time in their lives

The mycoses like tuberculosis show a particular predilection for the lungs There are many resemblances to tuberculosis clinically pathologically and immunologically Indeed this distinction is often quite difficult Other chronic lung diseases such as neoplasms are mimicked The competent internist has no choice but to equip himself with a working knowledge of how the mycoses can be diagnosed

The diagnosis of fungus infection may be definitively established by (1) direct microscopic demonstration of the organism in tissue exudates body fluids and (2) culturing of the organism Other adjunctive and less definitive procedures are animal inoculation and serologic tests For a complete discussion of these latter methods the reader is referred to several summaries [5-9]

The present paper will deal largely with the methods by which fungi may be recognized microscopically in tissue because (1) significant progress has recently been made in this field and (2) less experience and training is required to make accurate diagnoses The particular procedures to be used in any given case depend on the nature of the infection that is whether the disease is systemic or whether

it is restricted to the skin and its appendages The following synopsis is given to provide a background for comprehending the diagnostic problems

Fungus infections include perhaps the commonest, and the rarest of infectious diseases Ringworm of the feet is a vulgar disease affecting up to 60 per cent of adult males On the other hand most physicians have never seen a case of blastomycosis or disseminated coccidioidomycosis The clinical spectrum of fungus diseases is large Prognostically many of the cutaneous mycoses are trivial the systemic ones may be death dealing The fungus infections may be divided into two general classes (1) the superficial infections and (2) the systemic or deep mycoses These differ in respect to the causal species in the degree of tissue invasion in communicability, in immunologic response and most important, in prognosis

THE SUPERFICIAL FUNGUS INFECTIONS

The ringworms or tineas, are the commonest and the best known of this group They are caused by a group of related fungi which live in the keratinized portion of the hair skin and nails These fungi grow only in the dead, horny, stratum corneum of the epidermis and are incapable of invading tissues containing living cells They grow on the skin debris so to speak and never produce systemic disease Their parasitism is meager Microscopically the fungus in its parasitic state is made up of undifferentiated branching threads or filaments (hyphae) In its growth the fungus digests keratin an otherwise refractory substance On the glabrous skin the lesion extends centrifugally from the point of inoculation and usually has a round outline The hyphae tend to die out of the central area so that the border is seen to be the region of activity clinically Hence the peripheral area contains the greatest quantity of fungus Scrapings for diagnosis are collected from this region The hyphae of all the species which cause ringworm have a similar appearance in the stratum corneum Distinction is not possible microscopically Therefore identification of a particular species depend upon culturing of the organism There are difficulties since this latter procedure is often unsuccessful in known cases of ringworm In fact in only about 50 to 60 per cent of the cases of ringworm of the feet can the fungus be isolated On the other hand with thorough methods the fungus elements may be demonstrated microscopically in practically all cases This of course emphasizes the diagnostic value of direct microscopic tests in comparison to cultural methods As a matter of fact although isolation of the organism is desirable the failure to do so does not particularly compromise the clinical management of the case

When the scalp is involved as in tinea capitis the ringworm fungi grow within the keratinized portion of the hair as well as in the stratum corneum of the surface epidermis The bulb at the base of the hair root is not invaded since this contains living hair matrix cells The diagnosis of ringworm of the scalp presents little difficulty because of the enormous quantity of fungus elements concentrated in each infected hair Fungi are more difficult to detect in infected nails The hyphae occur not only in the nail plate itself but often subungually in the nail bed Because of the slow rate of nail growth the hyphal filaments are often in a degenerated state and are recognized with difficulty

The ringworm fungi are closely related morphologically and produce similar diseases They account for the majority of superficial fungus infections Certain

less common superficial mycoses are caused by a miscellaneous, unrelated group of organisms These organisms, too, are essentially saprophytes and live in the keratinized skin debris Unlike ringworm infections the diseases caused by these organisms are not particularly contagious and cultural isolation is either impossible or unnecessary for identification These diseases are (1) tinea versicolor, (2) erythrasma (caused by a bacteria like fungus Nocardia) (3) trichomycosis axillaris (a trivial disease of axillary hair caused by an actinomycete, (4) piedra (an unusual disease of the hair and beard) (5) tinea nigra Of this group only tinea versicolor and erythrasma are common enough in the United States to have much clinical significance Fortunately both are identifiable microscopically in skin scrapings The causative organism cannot be isolated in either case

The cardinal feature of all superficial fungus infections of the glabrous skin is scaling This is produced by the growth of the fungus in the stratum corneum with its consequent interference with the normal desquamation of keratinized cells

Moniliasis (caused by Candida albicans) is sometimes considered to be a superficial fungus infection Usually it is a disease of the mucous membranes (thrush, monilial vaginitis) or intertriginous areas of the skin Occasionally moniliasis is systemic Budding cells in addition to hyphae are seen in the lesions This morphologic combination (budding cells and hyphae) is diagnostic This organism has a higher degree of parasitism than the fungi described

Culturing of the Ringworm Fungi —These fungi are not fastidious in their nutritional requirements Practically any medium containing a hexose sugar and a source of peptone will support good growth The basic requirements of a suitable medium are that the colonies should be recognizable on initial isolation and that the nutritional conditions should not favor the growth of bacteria The chief source of difficulty is bacterial and mold contamination Various media have been devised to overcome this As a matter of practice however none of these have proved clearly superior to the following simple modification of the old Sabouraud agar

glucose	20 grams
neopeptone	10 grams
agar	18 grams
water	1 liter

The *p*H of this medium is about 5 The normal skin bacteria (principally Micrococci) grow poorly in this medium and ordinarily do not interfere with isolation Bacteria deriving from the gastrointestinal tract particularly species of Proteus are more troublesome and may overgrow the fungus Penicillin and streptomycin 25 to 50 units each per ml have been recommended to combat bacterial contamination[10] Twenty five to fifty units per ml of aureomycin terramycin or chloromycetin alone or in combination may be used Antibiotics have not however greatly increased the successful isolation of ringworm fungi

Mold contaminants are a more disturbing problem These are ubiquitous They grow rapidly and overwhelm the more slowly growing pathogens Raising the *p*H of the medium to 10 has been claimed to inhibit the growth of the common molds while permitting the pathogenic fungus to grow[11] This has not been widely adopted Littman has included oxgall in his medium in an attempt to limit the spread of mold contaminants[1] This medium also contains gentian violet and

obscures the recognition of pigment which may be valuable for identification. In addition the gross morphology of pathogens on this medium is so atypical as to render them non recognizable. Potato dextrose agar (Difco) is often used. Although several media may be used by mycologic specialists the simple medium given above is recommended for routine use. It is perfectly adequate for most purposes.

Pieces of nails or infected hairs may be washed for one to three minutes in 70 per cent alcohol before inoculating the tubes. Scaling lesions of the skin should be vigorously rubbed with an alcohol soaked cotton sponge before collecting the scrapings.

Microscopic Demonstration of Fungi in Superficial Fungus Lesions — Until recently, the time honored method of examining skin, hair and nail specimens for fungi involved mounting the material in a few drops of 10 per cent potassium hydroxide. After gentle heating and preferably after allowing the mount to clear for fifteen to thirty minutes the preparation was examined under the microscope. The strong alkali served to soften and clear the keratinized substrate containing the fungus hyphae. Because of the refractory cellulose chitin complexes in the fungus cell wall the hyphae resisted the destructive effect of the alkali and were thus rendered visible by contrast. The lack of color in such an unstained mount is the chief compromising feature of an otherwise simple and useful method. The untrained and the inexperienced often encounter difficulties in differentiating artifacts. For instance, the so called mosaic fungus which is really an amorphous crystalline material is often a source of confusion. Occasionally the walls of the cornified stratum corneum cells form patterns which resemble branching hyphae. Frequently inadequate specimens are collected which contain only 1 or 2 small hyphal fragments. The detection of these few fragments by a conscientious person may require fifteen to twenty minutes of microscopic examination of the unstained specimen. The examiner often has an uneasy doubt that the fungus may have been missed unless he spends this amount of time studying the slide thus while the potassium hydroxide mount has the great advantage of being easy to prepare the results with it are uncertain and time consuming in inexperienced hands. The potassium hydroxide method is however quite adequate for examining fungus infected hair because of the great abundance of fungus elements.

Various attempts have been made to develop a staining technique which would render the detection of fungi in skin scrapings less difficult. The procedures involving the use of methylene blue and polychrome methylene blue have given erratic results in the writer's hands[13,14]. The methylene blue stain seems to be adequate only when the specimen happens to include actively growing hyphae and when the scrapings are unusually thin. On the other hand the application of the Hotchkiss McManus stain (*cf* p 151) to this problem has been advantageous[1].

This latter stain enables the histochemical demonstration of substances belonging to the general class of carbohydrates[16,17]. According to Hotchkiss any complex polysaccharide will stain if it contains the 1–2 glycol grouping in unsubstituted form or the equivalent structure in which OH groups are replaced by amino or alkylamino groups. Although the chemical specificity has been challenged[18] it is evident that for the most part the substances which stain in tissue are polysaccharides. Thus normal components such as glycogen mucin, and hyaluronic

acid react strongly Obviously then, this stain cannot be regarded as uniquely specific for the fungus cell Strong staining of the fungus cell is to be expected from the composition of its cell wall This relatively tough structural material is a cellulose chitin complex Both of these components are polysaccharides

The essential steps in the Hotchkiss McManus procedure are hydrolysis of the tissue in 1 per cent periodic acid for about ten minutes followed by exposure to the Schiff reagent for approximately another ten minutes The Schiff reagent is simply decolorized basic fuchsin (the leuco form) an unstable compound In the presence of aldehydes the Schiff reagent is reconstituted to its original red color According to the theory (with which there are some difficulties) the aldehyde groupings are released from polysaccharides by the hydrolytic and oxidative action of periodic acid At the sites of aldehyde formation a red color will develop following reconstitution of the Schiff reagent to its original colored form Hence tissue polysaccharides stain red and the tissue background is either colorless or light pink It is not appropriate to detail further here the chemistry of this reaction It has recently been reviewed by DeLamater *et al* [19]

The brilliant staining of the cell wall of all the pathogenic fungi is a conspicuous feature of this procedure In addition the cytoplasm is rather intensely stained Evidently the cytoplasm of most fungus cells contains a higher concentration of carbohydrates than that of other microorganisms This seems to be a more or less characteristic histochemical feature of the fungus cell Although some bacteria will take up the stain, the dye is generally distributed as discrete masses or granules within the cell The cytoplasm generally stains lightly in comparison to the fungus cell or it fails to stain altogether There is evidence that the cell membrane of some bacteria is stained Further work with bacteria is indicated

Even though this is not a uniquely specific fungus stain it is for all practical purposes a differential stain that is fungus cells may be readily differentiated from tissue cells or other microorganisms Certain animal tissues such as the liver and the salivary gland contain a high concentration of polysaccharides They therefore stain red The interference in contrast presented by the staining of the tissue background is not as troublesome as might be supposed Glycogen for instance is present in the form of fine granules in the liver cells The fungus cell however is relatively large and the size contrast is great enough to permit recognition The average fungus cell is considerably larger than that of pathogenic bacteria

Studies have been underway in our laboratory to adapt this histochemical reaction to the particular purpose of demonstrating fungi in tissue The method to be described may be unsatisfactory for other purposes in view of the limited objective of this work Specifically an attempt has been made to develop a practical procedure that would be acceptable for routine mycologic work One of the disadvantages of the original staining technique as outlined by Hotchkiss and McManus was the required use of the Schiff reagent (decolorized basic fuchsin) This reagent is not readily prepared (it generally has to stand over night before it can be used and the last traces of color have to be removed by charcoal adsorption) In addition it is rather unstable In order to preserve it for any appreciable length of time it must be kept in an air tight container in the ice box When exposed to the air it tends to recolorize spontaneously Arzac has demonstrated that somewhat similar results can be obtained with non decolorized basic fuchsin [20] Arzac s modification is still a general stain for carbohydrates closely

simulating the original procedure except that ordinary basic fuchsin (a stable reagent) instead of its leuco form is employed There are, however chemical differences Suffice it to say here that 0 1 normal hydrochloric acid will quickly decolorize Schiff positive material stained by the original Hotchkiss McManus technique Arzac s modification however, is an acid fast stain which resists the decolorizing action of acids This property, as will be shown, is useful in increasing the contrast between fungus cells and host tissue

In the present work Arzac s technique has been still further modified in the direction of simplicity and practicality in accordance with the limited objective stated above This procedure is superior to the potassium hydroxide method of examining skin scrapings for fungi A recently completed study showed that the potassium hydroxide method failed to detect about 15 per cent of the positive cases A particularly desirable feature of the stain is its demonstrated usefulness with specimens (such as from nail ringworm) in which fungus elements are scarce The advantages of the stain are a higher percentage of positive diagnoses and the greater certainty of reporting negative findings Minimal laboratory facilities are required In particular this technique has been found valuable in the diagnosis of ringworm of the feet tinea corporis ringworm of the nails tinea versicolor and moniliasis The results are particularly brilliant with specimens from this latter disease The bacteria like fungus causing erythrasma is best stained with methylene blue

Method -The method of collecting epidermal scales is most important Preferably a drop or two of Mayer s albumin (purchasable from any supply house) is placed directly over the lesion This is then vigorously scraped with a blunt knife Material from nails should be similarly collected The albumin allows the scrapings to stick to the knife and also accomplishes the removal of the horny material in the form of macerated thin shavings Thin smears are essential for the best results The macerated material on the knife is finally transferred to the slide and rubbed over the surface so as to affect an even distribution The albumin also enables adherence of the material to the slide during the staining procedure Slides prepared in this way may be shipped by mail and stained without loss of brilliance weeks later if necessary If the specimens arriving at the laboratory consist of thick horny pieces or tops of vesicles these must be first pared down with a knife In this case the slide should be prepared before hand by smearing over it a drop of Mayer s albumin The scrapings are then rubbed over the slide as before It is far more desirable however for the individual collecting the scales to mount them directly onto the slide In suspected cases of moniliasis of the mouth or vagina the swab used to collect the sample is simply rubbed over an albuminized slide The following procedures are best carried out in a series of Coplin staining jars which may be permanently set up for routine use

1 Immerse for one minute in 95 per cent alcohol

2 Immerse for five minutes in a 5 per cent aqueous solution of periodic acid (obtainable from Eimer and Amend Co New York City)

3 Transfer to the following solution for two minutes

Basic fuchsin	0 1 gram
95% ethyl alcohol	5 cc
Water	95 cc

(Dissolve the basic fuchsin in the alcohol and then add the water)

4 Rinse briefly in tap water

5 Immerse in the following solution for ten minutes

Zinc hydrosulphite	1 0 gram
Tartaric acid	0 5 gram
Tap water	100 0 ml

Zinc hydrosulphite may be purchased from the Virginia Smelting Company W Norfolk Virginia The solution is stable for at least one month if kept in a closed jar It may be used repeatedly It may be conveniently kept in a stoppered bottle and poured into a Coplin jar before use or a screw cap type Coplin jar may be employed Zinc hydrosulphite is a whitish powder readily soluble in water In the presence of acid sulfur dioxide (the active agent), is released The freshly prepared solution contains finely dispersed sulfur which however, does not interfere with its immediate use Within twenty four hours the sulfur will have settled to the bottom leaving a clear solution Removal of the sulfur by filtration is not required The solution is usable as long as it continues to have a strong smell of sulfur dioxide

6 Rinse briefly in water
7 Immerse for two minutes in saturated aqueous solution of picric acid
8 Rinse briefly in tap water
9 Add 1 or 2 drops of glycerin and cover with a cover slip

Glycerin mounted slides are satisfactory for routine use and can be kept for at least several days If semi permanent preparations are desired the specimen may be mounted directly after Step 8 in a drop or two of polyvinyl alcohol mounting medium [1] If on the other hand completely permanent mounts are desired it is first necessary to dehydrate the preparations after Step 8 This may be accomplished by immersing in 95 per cent alcohol absolute alcohol, xylol and a final xylol solution each for 1 minute Following this the preparation may be mounted in clarite or HSR mounting medium Such preparations may be filed The glycerin mount is recommended for ordinary use and is simplest

The fungus elements are stained red Splendid color contrast is afforded by the yellow stained keratin background The brilliance of the stained preparation depends upon the thickness of the smear and the age of the hyphae Old partially degenerated hyphae stain poorly Because of the impenetrability of keratin the fungi in thick pieces of horny material are poorly stained Inspection for the presence of fungi may be done under the low power of the microscope Scanning the slide for a few minutes will determine whether or not a fungus is present This method is far more tedious and time consuming than the potassium hydroxide technique Its advantages however overweigh these considerations Actually time may be saved in the long run since only a few minutes are required to inspect the slide A negative result with this method is much more meaningful than with the KOH technique

Staining of Fungi in Culture —The above staining technique is also useful for morphologic studies in vitro The microscopic characteristics of the various pathogens may be brilliantly demonstrated in a permanent mount if the fungus is first grown on a cover slip Such specimens make excellent demonstrations for class preparations or for photographs Inspection of microscopic features particularly the type and habit of spore formation is essential for distinguishing a fungus species There are several ways of achieving this Perhaps the simplest and the most effective is as follows using a sterile unlipped test tube 12 mm in

width the mouth of which has been flamed (1) cut 3 or 4 cylinders of agar out of a petri dish containing about 30 ml of the culture medium given above The cylinders should be approximately equidistant from each other Each cylinder is then fished out with a needle as gently as possible and simply laid on the agar surface next to the excavitation The plate thus contains 3 or 4 agar cylinders immediately adjacent to each hole A crude suspension of the fungus to be studied is prepared by adding about 2 ml of sterile saline to an agar test tube culture and scraping the surface with a needle The suspension is then drawn into a 1 ml pipette and a single drop released onto the top of each agar cylinder Following this a clean unsterile cover slip 20 to 22 mm square is flamed gently and dropped over the agar disc in such a way that the center of the disc is equidistant from the 4 corners of the cover slip The fungus grows on the upper surface of the agar cylinder and extends radially outwards on the underside of the cover slip A relatively thin layer of growth is formed peripheral to the agar cylinder Advantageously the fungus threads grow quite closely appressed to the glass in the complete absence of medium This growth habit greatly facilitates staining mounting and visualization Moreover, sporulation is favored Such a slip on which a fungus has been growing from one to three weeks may be stained in exactly the same manner as skin scrapings The preparation may be processed as soon as characteristic spores have developed Abundant growth is undesirable Because of the thinness of the preparations and the fact that the fungi are not embedded in tissue, the period in the zinc hydrosulphite solution should be reduced to one minute Counterstaining with picric acid is unnecessary Naturally for permanent mounts the slip must be dehydrated and processed as described above Small staining jars are available for the processing of cover slips five to ten at a time This method of growing the fungus on a cover slip is a simplified modification of the one described by Riddell [29]

THE DEEP OR SYSTEMIC MYCOSES

Many of the fungi which cause systemic disease live saprophytically in the soil A variety of unrelated fungi have been incriminated It is a peculiarity of some of these organisms that their microscopic appearance in tissue is unlike their *morphology in culture* Such *organisms are called dimorphic* The organisms causing histoplasmosis blastomycosis sporotrichosis coccidioidomycosis and others are in this group Usually round cells which may bud are found in tissue whereas mold like growths composed of branching hyphae and reproductive spores are developed on culture media at room temperature The tissue phase because it is usually made up of budding cells (with the exception of Coccidioides) is referred to as the yeast phase The systemic mycoses often occur in conjunction with chronic debilitating diseases such as tuberculosis and the lymphomas They are often fatal in the disseminated form Histopathologically the tissue reaction may be quite similar to that of other granulomas Definitive diagnosis requires the demonstration of the organism

Culturing of the Organisms Causing the Systemic Mycoses — The usual materials for culture consist of biopsy specimens sputum and body fluids With contaminated specimens such as sputum or exudates it is advantageous to incorporate 50 micrograms per ml of one of the newer antibiotics with a broad antibacterial spectrum (aureomycin chloromycetin terramycin) Combinations of

these are practical and in some instances desirable The bacteria like fungi causing actinomycosis and nocardiosis are susceptible to these antibiotics When these are suspected antibiotics cannot be incorporated in the isolation medium It is customary in attempting to isolate pathogenic fungi to inoculate at least 2 plates, one of which contains a blood medium and is incubated at 37° C and the other of which contains a Sabouraud like medium (such as the one given above for the ringworm fungi) and is incubated at room temperature In some laboratories only two blood agar plates one of which contains an antibiotic, are inoculated and these are incubated at 30° C This latter is possibly the preferred procedure The blood agar is conveniently made up from dehydrated Difco blood agar base with 5 to 8 per cent of human or horse blood added The addition of 1 per cent dextrose to this medium enhances the growth of fungi Further details for isolating the systemic fungi will be found elsewhere [3]

The Staining of Fungi Causing the Systemic Mycoses —The pathogenic fungi as a group are said to be uniformly gram positive This property is so diminutive in most instances as to be of little diagnostic value The routine hematoxylin and eosin stain was generally preferred for staining most fungi in tissue until the value of the Hotchkiss McManus stain was demonstrated With this latter stain, the study of the morphology of fungi in tissue has been greatly enhanced Because this stain is so definitive for fungi it has been possible to see forms in tissue which were but poorly, or not at all visualized before Some previous notions had to be corrected The so called capsules of Histoplasma capsulatum and Sporotrichum schenckii seen in hematoxylin and eosin preparations have been found to be artifacts [3] In our hands the results with the modification to be described have been superior to those obtained with the Hotchkiss McManus technique More intense staining of the fungus cell is achieved

Biopsy specimens may be handled in the following way The tissue may be fixed in any of the routine fixatives They may then be processed embedded in paraffin and sectioned in the conventional manner After deparaffinizing in xylol, the slides are immersed in absolute alcohol and finally washed in distilled water Two slides should be simultaneously treated as follows

1 Immerse in 1 per cent periodic acid ten minutes
2 Rinse in tap water
3 Immerse in the following solution two minutes

Basic fuchsin	0 1 gram
95% absolute alcohol	5 cc
Water	95 cc

4 Rinse in tap water
5 One slide is treated for ten minutes and the other for thirty minutes in the following solution

Zinc hydrosulphite	1 0 gram
Tartaric acid	0 5 gram
Water	100 ml

It has been found that various species of fungi react differently to the zinc hydrosulphite solution One of the two slides will show an appropriate amount of differentiation For instance, the organism causing sporotrichosis tends to be overstained by this procedure so that although the ten minute slide is acceptable the contrast between the organism and the tissue is far better with the thirty

minute exposure. In general, better results are obtained with the thirty minute procedure.

6. Rinse in tap water for one minute.
7. Counterstain in saturated aqueous solution of picric acid for two minutes.
8. *Rinse briefly in tap water.*
9. Dehydrate, clear and mount in the usual way.

The fungi stain bright red or various shades of magenta. The tissue background takes up a variable amount of the yellow picric acid depending upon the tissue itself. It will be either quite yellow, which affords the best contrast, or a reddish yellow which is less desirable. Increasing the exposure to the zinc hydrosulfite solution helps remove the red staining of the background and improves the yellow counterstain. Occasionally a three hour exposure in zinc hydrosulfite solution gives optimal results. With this technique the least satisfactory results are obtained with *sections containing Histoplasma capsulatum, the smallest of the pathogenic fungi. Considerable improvement is secured by treating the* specimen for one hour at 37° C in 1 per cent periodic acid solution instead of ten minutes.

Hematoxylin and eosin stained slides may be immediately restained by this method without any further manipulation than the removal of the cover slip with xylol and the rehydration of the specimen. Following this the slide is put into 1 per cent periodic acid for ten minutes as usual (this removes the hematoxylin and eosin stain) and the rest of the procedure is as given above. Consequently if one suspects a hematoxylin and eosin stained granuloma to be of mycotic origin and fungus cells are not detectable the specimen may be restained without making another section.

The Staining of Body Fluids, Exudates and Sputum.—Body fluids such as spinal fluid, urine, and pleural effusion fluid are easily handled. Preferably *the specimen should be centrifuged and the sediment spread over an albuminized* slide. The preparation is then treated with 1 per cent periodic acid for ten minutes, followed by the basic fuchsin solution for two minutes and exposure to the hydrosulphite solution for one minute. It is then counterstained with picric acid for two minutes.

Difficulties arise in the staining of sputum. This material contains a large quantity of muco polysaccharides which of course take up the stain. Thus the background material will be stained a more intense degree of red than is desired. Some improvement in the contrast between the fungus cells and the polysaccharides in the sputum can be obtained by prior hydrolysis of the sputum in 10 per cent sulfuric acid solution. The sputum is mixed with an equal quantity of 10 per cent sulfuric acid and incubated for four hours at 37° C. The mixture is then centrifuged and the sediment spread thinly over an albuminized slide. The slide is then stained as for spinal fluid or urine. Although this method of examining sputum is useful and preparations are better than any other available procedure it leaves much to be desired.

It is expected that the routine use of this stain for diagnostic purposes will facilitate the recognition of fungus containing tissues and fluids by those with no special training in mycology. The stain is definitive enough so that even the inexperienced will generally be able to recognize a fungus cell that he may never have seen before. Fortunately the organisms causing the systemic mycoses have distinctive characteristics whereby they can be identified in tissue. Thus even

in the absence of positive cultures a diagnosis can usually be made if the proper material for staining is available After one has established that fungi are present in the preparation, consultation with the standard textbooks or with a mycologist should be sought The tissue phases of some of the systemic organisms may resemble each other in part but an intensive search by the informed observer will generally reveal some structure which is diagnostic of the species

An examination of the accompanying illustrations (Figs 19-23) will show the characteristic features of most of the organisms responsible for human mycotic diseases

FIG 19 —1 *Biopsy from a case of human tinea corporis (ringworm of the smooth skin)* Periodic acid fuchsin stain counterstained with hematoxylin × 110 The segments of strongly stained branching hyphae may be seen among the loose keratinized scales of the stratum corneum It is to be noted that the fungus threads are entirely confined to this dead horny layer and are unable to penetrate down into the living portion of the epidermis All the species of ringworm produce this same picture

2 *Scrapings from a case of tinea versicolor* Periodic acid fuchsin stain × 248 The hyphal segments and the grape like cluster of budding cells make up a diagnostic combination The round budding cells may actually be another fungus perhaps a species of Pityrosporum which regularly associates itself with the organism causing tinea versicolor

3 *Scrapings from a case of human athlete s foot* Periodic acid fuchsin stain × 248 The ramifying branching fungus threads are diagnostic of a ringworm infection The fungi are rarely this abundant

4 *Skin scrapings from a case of experimental ringworm in the guinea pig* Periodic acid fuchsin stain × 193 The appearance is quite similar to human ringworm

5 *Biopsy from a case of tinea capitis due to Microsporum audouini* Periodic acid fuchsin stain counterstained with hematoxylin × 138 The section shows an infected hair emerging from a follicle Some of the hyphae which are responsible for the destruction of the hair may be seen within it The most diagnostic feature however is the mosaic of round spores clinging in masses around the hair

6 *Biopsy from a case of tinea capitis due to Trichophyton tonsurans* Periodic acid fuchsin stain counterstained with hematoxylin × 275 The section shows a hair whose internal substance i occupied by chains of rectangular fungus cells These chains of cells distingui h Trichophyton infections from those due to species of Mi rosporum Note that in this case there are no spores around the hair

7 *A culture mount f Microsporum fulvum* Periodi acid fuchsin stain × 124 This culture was grown on a cover slip The boat shaped structures are multi septate spores They are diagnosti of this spe ies

8 *A culture mount of Trichophyton mentagrophytes* Periodi acid fuchsin stain × 138 This culture was also grown on a cover slip Note the hyphal branches adorned with round to pyriform spores Such beautiful preparations in whi h the spores remain intact on the spore bearing branches can only be obtained by growing the culture on a slip or slide

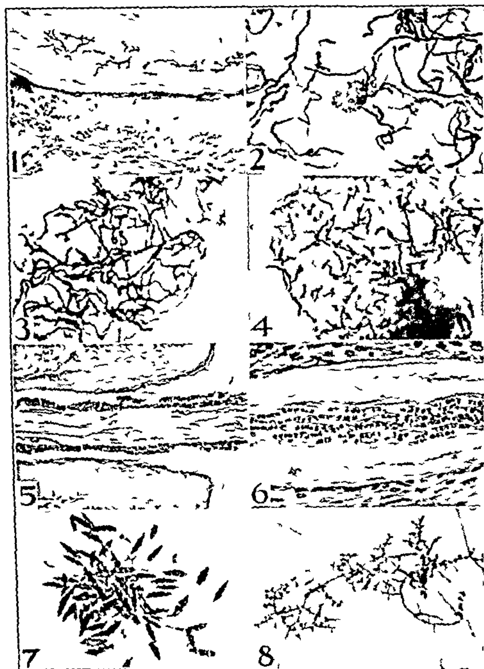


FIG 19 (*Legend on opposite page*)

Fig 20—1 *Biopsy from a human case of blastomycosis (lung)* Periodic acid fuchsin stain × 193 Note how densely the cell wall stains In addition in this particular organism the nuclei are remarkably well stained This has not been noted for other fungi

2 *Biopsy from a mouse with blastomycosis (peritoneal nodule)* Periodic acid fuchsin stain × 138 Note that some of the cells are in the process of budding These large round budding cells allow a definitive diagnosis to be made

3 *Same section as No 2 from a mouse with blastomycosis* Hematoxylin and eosin stain × 83 This section is loaded with organisms Yet with the hematoxylin and eosin stain these are difficult to pick out among the inflammatory cells of the host The great virtue of the periodic acid fuchsin stain is that the background remains colorless In skin blastomycosis the sections often contain only one or two fungus cells These are extremely difficult to detect with ordinary stains

4 *Biopsy from a human case of histoplasmosis (spleen)* Periodic acid fuchsin stain × 385 The organisms appear as round cells which occasionally bud Note that no capsule is evident here

5 *Biopsy from another human case of histoplasmosis (lymph node)* Periodic acid fuchsin stain × 495 The organisms are in a cluster within a host macrophage which of course does not stain This is the usual appearance of the organisms with this stain One observes a dark concentrated mass centrally or eccentrically placed within the cell This represents cytoplasm which has retracted away from the cell wall leaving a space The cell wall is clearly visible around this space This space has been mistaken for a capsule because of the failure of the cell wall to stain with ordinary methods The capsule is an artefact

6 *Biopsy of the chick chorio allantoic membrane infected with Histoplasma capsulatum* Hematoxylin and eosin × 220 Note the macrophages loaded with what appear to be capsulated organism Usually the organisms do not stain even this well with hematoxylin

7 *Biopsy from a human case of histoplasmosis (adrenal)* Periodic acid fuchsin stain × 358 The organisms again appear to have a capsule but in the actual specimen one could clearly see the cell wall surrounding the apparent capsule The Histoplasma organisms shown in No 4 were prepared in such a way as to avoid shrinkage of the cytoplasm Hence the appearance of a capsule was avoided

8 *Culture mount of Histoplasma capsulatum* Periodic acid fuchsin stain × 138 The round pores ornamented thickly with club shaped protuberances are diagnostic of this species



FIG 20 (*Legend on opposite page*)

FIG 21 — 1 *Biopsy from a mouse with sporotrichosis (peritoneal nodule)* Periodic acid fuchsin stain × 275 This organism is usually described as being cigar shaped Actually however when one can observe it against a non stained background as in this specimen multiplicity of forms become visible Some of the cells are producing rod shaped buds This is rather typical Many of the organisms however are quite round and large This variation in size and shape is more characteristic than are the so called cigar shaped figures

2 *Biopsy from a human case of sporotrichosis (lung)* Periodic acid fuchsin stain × 359 This organism like Histoplasma capsulatum which it may strongly resemble is ordinarily intracellular Therefore in the early stages of infection it may be found distributed in tight clusters within macrophages This was a case of human lung infection in which again one may note the variability in the morphology of the organisms

3 *Biopsy from a mouse with sporotrichosis (spleen)* Hematoxylin and eosin stain × 440 This is an unusual photograph showing masses of fungus cell within macrophages These appear to be encapsulated much like Histoplasma capsulatum This too is an artefact due to retraction of the cytoplasm away from the cell wall

4 *Biopsy from the chick chorio allantoic membrane infected with Sporotrichum schenckii* Hematoxylin and eosin stain × 220 Note that the intracellular fungi appear to have very definite capsules

5 *Biopsy of human skin containing a sporotrichotic abscess* Periodic acid fuchsin stain × 55 This low power view will illustrate how readily the organism may be visually localized in the tissue All of the darkly stained material represents organisms These would not be detectable at this power with the hematoxylin and eosin stain The organisms are rarely this numerous in cutaneous sporotrichosis

6 *Biopsy from the chick chorio allantoic membrane infected with Sporotrichum schenckii* Periodic acid fuchsin stain × 303 Note that the capsule evident in photo 4 have disappeared with *this stain Again there is a great variability in shape*

7 *Biopsy from the human brain containing Sporotrichum schenckii* Periodic acid fuchsin stain × 275 The organisms are intracellular as is indicated by their being aggregated in clusters Note that there is no capsule and that except for their being distinctly elliptical there is a striking resemblance to Histoplasma capsulatum In certain cases this morphologic cell type appears to be characteristic of human sporotrichosis when the lesions are in the viscera rather than the skin

8 *Scrapings from a case of mucocutaneous moniliasis* Periodic acid fuchsin stain × 165 These structures are all artefacts although they take up the stain strongly One must note their irregularity and the lack of a clear cut wall Such artefacts are uncommon



Fig 21 (*Legend on opposite page*)

FIG 22—1 *Spinal fluid smear from a case of human torulosis* Periodic acid fuchsin stain × 220 This organism (Cryptococcus neoformans) has a distinct wide capsule which is well shown The budding round capsulated cells when found in spinal fluid are diagnostic of torulosis

2 *Biopsy from a human case of torulosis (kidney)* Periodic acid fuchsin stain × 110 Note how easily the organisms may be seen against the tissue background Most of the capsular material has dissolved out in the processing of the tissue leaving a small cyst like space in which the organisms are suspended

3 *Scrapings from a case of thrush (moniliasis)* Periodic acid fuchsin stain × 193 The combination of hyphal threads and budding yeast cells is diagnostic of this organism (Candida albicans)

4 *Scrapings from a case of moniliasis of the nails* Periodic acid fuchsin stain × 193 The appearance is similar to that observable in photo 3 This yeast like organism is often very difficult to detect in infected nails with the potassium hydroxide method

5 *Biopsy from a rabbit kidney infected with Candida albicans* Periodic acid fuchsin stain × 165 The organisms stand out starkly This picture would have been completely obscured had the specimen been stained with hematoxylin and eosin since the organisms are lying in the midst of a dense collection of inflammatory cells Note again the hyphae from which budding structures are being formed

6 *Biopsy from a mouse kidney infected with Candida albicans* Periodic acid fuchsin stain × 220 The diagnostic combination of budding cells and hyphal fragments is again in evidence In this case not even the outline of the background tissue shows up

7 *Scrapings from the normal human scalp* Periodic acid fuchsin stain × 303 This organism is Pityrosporum ovale It is a normal inhabitant of the skin This organism tends to occur in the sebaceous skin areas because lipoids are needed in its nutrition It should not be confused with the organism causing moniliasis

8 *Scrapings from the normal scalp* Periodic acid fuchsin stain × 358 The organism shown is another lipophylic fungus probably Pityrosporum orbiculare This organism could be more easily confused with Candida albicans and even with Cryptococcus neoformans It is a normal skin inhabitant



FIG 22 (*Legend on opposite page*)

Fig 23—1 *Biopsy from a human case of coccidioidomycosis (kidney)* Periodic acid fuchsin stain × 165 In addition to the mass of endospores escaping from a ruptured spherule one may see here and there in the tissue round fungus cells of varying size Most of these latter cells are not visible in hematoxylin and eosin preparations Consequently the organisms in this disease are far more numerous in tissue than previously suspected

2 *Biopsy from a case of human coccidioidomycosis (skin)* *Hematoxylin and eosin stain × 165* One may note a spherule with vaguely staining enclosed endospores This is surrounded by a mass of inflammatory cells Although a few round fungus cells are present within this inflammatory collection they cannot be distinguished

3 *Biopsy from a case of human coccidioidomycosis (skin)* Periodic acid fuchsin stain × 330 This spherule is packed full of endospores Strangely enough the wall of the spherule stains poorly

4 *Biopsy from a mouse lung infected with Coccidioides immitis* Periodic acid fuchsin stain × 193 In addition to the cluster of cells indicating a spherule one may also note an enormous number of round cells of varying size Very occasionally some of these will appear to be budding

5 *Culture mount of Coccidioides immitis* Periodic acid fuchsin stain × 275 The rectangular spores arranged alternately in a chain are diagnostic Every other cell is a viable spore while the cells in between dry up and become ghosts

6 *Biopsy from a human case of coccidioidomycosis (brain)* *Periodic acid fuchsin stain × 358* One may note here the variation in the size of the fungus cell

7 *Biopsy from a human case of South American blastomycosis (lung)* Periodic acid fuchsin stain × 248 The organism is composed of large budding cells in which one may clearly see that the cytoplasm has retracted away from the cell wall Quite characteristically one may observe much smaller fungus cells which initially were produced as small buds around the rim of the large cell Several of these small round cells which appear to have capsules are evident in this section These latter structures generally cannot be seen with hematoxylin and eosin stained specimens

8 *Biopsy from a human case of South American blastomycosis (lung)* Hematoxylin and eosin stain × 193 This section contains a great many fungus cells which are obscured in the inflammatory infiltration The small round cells visible in the previous section are of course completely non detectable



FIG 23 (*Legend on opposite page*)

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Chapter

5

THE LIVER

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THE last decade has shown giant strides in our knowledge of the pathogenesis of many diseases of the liver It has of course been a decade characterized by exceptionally rapid advances in our knowledge of many fields of medicine This may in some ways be regarded as one of the indirect benefits of war The intense mobilization of the resources and manpower of many nations to solve rapidly problems which have military importance permits a rate of progress which is difficult under peacetime conditions

In recent times some of the methods of work, found so valuable during war, have been continued in times of relative peace One of the most important of these is that, if groups of individuals interested in the same problems but approaching them from different points of view can be gotten together in conferences and symposia the discussion so provoked allows the individuals to reassess their problems and reach a more rapid and satisfactory solution of them This is understandable merely from the standpoint that it gives an opportunity to pool experiences materials and ideas

Cooperative efforts of this type have been numerous during the past decade in relation to studies on the liver During the war due to the prevalence of viral hepatitis in the American and British troops the resources of the Army Medical Corps and the Army Institute of Pathology were to a large extent concentrated on a study of the etiology and pathogenesis of this condition This work has been continued and broadened in recent years by a series of conferences sponsored by the Josiah Macy Jr Foundation[68–73] and a Ciba Foundation[17] symposium in 1951

The progress has been so great during the decade that any attempt to survey the entire field of liver pathology would require such a cursory treatment as to be completely valueless Some of the more important features have been selected therefore because these seem to be of more than casual interest and importance It is planned to take up the following subjects in the order listed

1 Histochemistry
2 Function tests
3 Biopsy
4 Newer concepts of normal and abnormal blood circulation
5 Viral hepatitis
6 Fatty infiltration
7 Ceroid
8 Necroses

9 Cirrhosis
  (a) Classification
  (b) Healed acute yellow atrophy (Focal postnecrotic cirrhosis)
  (c) Alcoholic cirrhosis (Diffuse fatty [nutritional] cirrhosis)
  (d) Pigment cirrhosis
  (e) Biliary cirrhosis
  (f) Syphilitic cirrhosis

10 Regeneration

11 Primary cancer

HISTOCHEMISTRY

Although the last decade has witnessed significant advances in our insight into the participation of the liver in a variety of biochemical processes[1] there has been no concomitant expansion of correlated histochemical studies of pathological changes in diseases of the liver Thus while numerous articles devoted to histo and cytochemical investigations have appeared between 1941 and 1951 only a small minority discuss applications of established histochemical methods to studies in histopathology the majority dealing with the development of new methods and techniques Because of this fact and the relative paucity of well substantiated studies pertaining to histochemical investigations of liver disease it is felt that a brief mention of trends in this field is all that is indicated at present

There have been three major approaches to the problem of intracellular localization of chemical substances of biochemical origin or significance

The first approach currently the most popular with cytologists and pathologists is based upon a group of methods in which a sliced tissue section (fixed or fresh) is subjected to a chemical reaction that is (a) specific for a single chemical group or compound and (b) leads to the formation of a stable, relatively insoluble colored compound which may be identified under the ordinary light microscope[40,113] This approach because of the relative simplicity of the apparatus required and techniques utilized will probably continue in vogue However many of the methods in this first group have had inadequate correlative studies with methods (to be discussed) which are more accurate and sensitive although technically more complicated[18,80,128] A further criticism is that quantitative studies do not appear readily feasible by methods in this first group

Ancillary to the above are methods which depend upon enzyme hydrolysis of the inter or intracellular constituents being studied in the section Comparison with control sections theoretically permits a qualitative cytologic identification of the compound in question

A second major approach utilizing visual examination of the tissue studied is by employing the absorption of ultraviolet light by tissue sections This depends upon a thorough knowledge of the absorption characteristics of the biochemical compounds under investigation and therefore is limited to those compounds which have been previously isolated in pure state and thoroughly identified by ultraviolet spectrophotometry Although superior to methods of the first approach both as regards quantitative evaluation and study of living cytoplasm ultraviolet microscopy has been limited in application by the average pathologist because of the cost of equipment technical knowledge required and relatively

small number of biochemical compounds adequately characterized by ultraviolet spectophotometry [15,40]

The third major approach is one which has been used primarily by the biochemists [18,1,8] It involves the homogenization and subsequent centrifugation of an aliquot of tissue samples of different particle size and density being isolated by centrifugation These may then be analyzed chemically and identified visually correlation thus being made between particulate cytological elements seen on tissue section and the particles isolated by centrifugation This method although (a) at present limited to such relatively homogeneous tissues as liver and (b) apparently incapable of furnishing a means of cytochemical identification of nonparticulate structures nevertheless seems to offer a precise and quantitative approach to a correlation of many histochemical and histopathologic changes involving particulate elements

Ultimately (and ideally) techniques involving microdissection and microanalysis of samples of cytoplasmic constituents and intercellular substances will offer the soundest basis for histochemical studies [40,6] That these relatively esoteric methods will ever enjoy widespread use appears unlikely However, they will probably serve an invaluable function in control studies of results obtained by methods in other categories since their accuracy and sensitivity as regards identification and analysis of cytologic constituents (particulate and nonparticulate) appear to be superior

Brief mention should be made of autoradiographic methods[37] and fluorescent microscopy[40,114] as approaches which should contribute progressively more to histochemical studies

In summary it is to be anticipated that with the continued application of physics to biological problems new techniques and instruments will appear leading to more exact histochemical procedures It is apparent that even without these the use of a wide variety of currently known histochemical techniques will greatly accelerate our understanding of the relationships between the histopathologic pathophysiologic and pathogenetic aspects of liver disease

LIVER FUNCTION TESTS

In recent years many new liver function tests[100] have been developed and the value of the older ones reassessed In reviewing the literature on them one is forced to the conclusion that although they are of undoubted value this value is a definitely limited one In his brief summary on Liver Function Tests in his Lowell Institute Lectures Himsworth[61] concludes with the statement that as yet there is no test which approaches in value a careful clinical assessment of the patient Nevertheless Liver Function Tests constitute quite a heavy portion of the work load of clinical laboratories in active particularly teaching hospitals

To quote Himsworth[61] again the tests can be regarded as a means of demonstrating the absence of liver damage or as a means of recognizing the nature of the damage present On the whole they can be divided into three main categories (1) Tests of excretory capacity (2) Tests of ability to synthesize and (3) a group of empirical tests for liver damage

The following are some of the more important function tests with an estimation of their value and limitations as discussed by Ham[47] in 1950

(1) **Bromsulfalein (BSP) Test.**—This depends upon the fact that, when the dye is injected intravenously, it remains almost completely in the blood stream until it is removed in a progressive manner (apparently, by the parenchymal cells of the liver), excreted into the bile, and eliminated in the feces. The normal value for the injection of 5 mg/kg is less than 5 per cent retention in the blood stream forty-five minutes after injection. Values of retention greater than 5 per cent are roughly proportional to the extent of liver dysfunction, if the patient's general medical condition is normal and there is no obstruction to the biliary system. Retention may occur in the absence of chronic liver disease in patients with hemorrhage, shock, cardiac failure, trauma, operative procedures, certain febrile illnesses, or febrile and nonfebrile states following injection of pyrogenic material.

It is considered one of the most sensitive and reliable tests detecting minimal or early impairment of the function of the liver parenchyma as well as demonstrating advanced liver damage. Retention suggests parenchymal damage or extrahepatic biliary obstruction.

(2) **Hippuric Acid Test.**—Benzoic acid is administered by mouth or intravenously, and the amount of hippuric acid excreted in the urine is measured. The formation of hippuric acid from glycine and benzoic acid is a function of the liver. In contrast to the BSP test, the hippuric acid does not depend on the patency of the biliary system. The intravenous test can be reproduced more easily and is more sensitive than the oral test. Considerable liver damage must occur before it is positive.

(3) **Level of Prothrombin, Response to Vitamin K.**—A failure of the prothrombin level to respond to vitamin K administered parenterally is important evidence of hepatocellular damage, but it does not indicate the type of damage. Since the prothrombin level of plasma may be decreased when other tests of liver function are normal, it should be determined whenever disease of the liver or biliary system is suspected, especially if surgical procedures are contemplated.

(4) **Galactose Tolerance and Clearance Tests.**—These test the ability of the liver to metabolize carbohydrate, *i.e.*, galactose. The galactose tolerance test is an oral test in which the galactose ingested by the patient and the galactose excreted in the urine during five hours is determined quantitatively. The galactose clearance is an intravenous test. The galactose is given intravenously and after seventy-five minutes the amount of galactose in the venous blood is determined quantitatively. The intravenous test is the more accurate.

Technically, the chemistries are somewhat difficult. These galactose tests are similar in importance to the BSP and the hippuric acid tests. The BSP is, however, the most sensitive of the three.

(5) **Total Cholesterol, Cholesterol Esters.**—In acute hepatocellular disease and diffuse fatty (nutritional) cirrhosis of the liver, the cholesterol is usually normal or below normal irrespective of the bilirubinemia. The cholesterol esters may decrease out of proportion to the decrease in cholesterol. The determination of cholesterol and cholesterol esters has significant limitations as a test of function of the liver and biliary system since changes may occur in other diseases that do not affect these structures primarily.

(6) **Bilirubin-Excretion Test.**—Bilirubin is injected and the amount excreted tested. The test is apparently more sensitive than the BSP test in detecting dysfunction of the liver parenchyma in the non-jaundiced patient. This test may be

the only test that is abnormal in patients with constitutional hepatic dysfunction

(7) **Plasma Proteins** —Chronic parenchymal disease of the liver is frequently associated with decreased albumin levels In acute yellow atrophy plasma fibrinogen may decrease below normal to such a degree as to produce hemorrhagic manifestations

(8) **Stability Reactions of Blood Serum** —These are the tests referred to by Himsworth[61] as empirical tests They depend on the production of flocculation or turbidity of a combination of serum with reagents, such as an emulsion of cholesterol and cephalin a buffered solution of the phenolic compound thymol a solution of a dye, colloidal red solutions of heavy metals such as colloidal gold or mercuric chloride in the Takata Ara reaction

(*a*) *Cephalin cholesterol Flocculation Test of Hanger* —Results are recorded from 0 to 4 plus A 3 plus or 4 plus reaction is distinctly abnormal and consistent with but not neccessarily diagnostic of damage to liver cells Positive tests may occur in atypical virus pneumonia malaria syphilis infectious mononucleosis and congestive heart failure The test takes forty eight hours to do

(*b*) *Thymol Turbidity and Flocculation Tests* —In the turbidity test a thymol barbital buffer solution is added to the serum In one half hour the turbidity is determined by comparing the specimen tested with standards The results are recorded in units Zero to 4 units are considered normal The *pH* of the reagents used is important False positives are sometimes found in rheumatoid arthritis virus pneumonia heart failure and infectious mononucleosis

The flocculation test is an adjunct to the turbidity test The tube is allowed to stand overnight and the flocculation read 0 to 4 plus Zero to 1 plus is considered normal

(*c*) *Colloidal Red Test* —The serum is mixed with a buffered solution of Scharlach R or Sudan IV and the flocculation produced is read after twenty four hours

(*d*) *Colloidal Gold Reaction* —Serum is mixed with colloidal gold The colloidal gold solution is added to serial dilutions of serum and the tubes are read after twelve to twenty four hours

(*e*) *Takata Ara Reaction* —The serum is diluted and alkalinized and a dilute solution of mercuric chloride added The mixture stands from twelve to twenty four hours and the flocculation is read in six dilutions

Ham[47] gives a discussion of the limitations and interpretations of the above tests The cephalin cholesterol flocculation test is a sensitive screening test It indicates incipient or occult damage to liver cells in patients with or without jaundice In viral hepatitis it is positive as long as active disease of liver cells is present It does not indicate quantitatively the degree of liver impairment in hepatitis or cirrhosis A positive test with obstructive jaundice suggests damage to liver cells

The thymol turbidity and flocculation tests are positive in more than 90 per cent of cases of infectious hepatitis They are usually negative in jaundice due to extrahepatic obstruction In infectious hepatitis positive thymol turbidity and flocculation tests persist longer than positive cephalin flocculation tests Thymol tests are technically more satisfactory than cephalin flocculation tests

In infectious hepatitis Neefe and Reinhold[108] found the cholesterol flocculation and thymol turbidity tests sensitive indices of the onset and the remissions of hepatitis In non icteric chronic hepatitis produced in volunteers Neefe[109] found the thymol turbidity test and the colloidal gold tests abnormal when many other

tests including the cephalin flocculation test, were normal. The colloidal gold test is perhaps more sensitive in certain instances of hepatitis. It is not, however, widely used.

The Takata Ara test is positive in a higher incidence in cirrhosis and hepatitis than in diseases in which the liver is not involved.

In general positive findings occur in those cases in which there is elevation of serum globulin or decrease in serum albumin or both.

(9) **Alkaline Phosphatase of Serum.**—Gutman and collaborators[4] have studied the alkaline phosphatase of the serum, determined by the Bodansky method, in patients with and without disease of the liver and biliary system. In 90 per cent of cases with obstruction of the common bile duct, serum phosphatase levels were more than ten Bodansky units. A similar percentage of patients with "catarrhal" jaundice had less than ten Bodansky units. The serum phosphatase was normal in 13 cases of hemolytic jaundice. It may be significantly elevated in chronic cholangiolitic hepatitis. The mechanism of this phenomenon is not clear.

The reactions of a man as experienced in liver disease to these function tests are worth quoting. Dr. Himsworth feels that (1) the presence of jaundice is a sufficient indication of the inability of the liver to excrete. (2) The bromsulfalein test is a useful index of the progress of the disease or for establishing recovery. (3) A low level of plasma albumin in a patient who has signs of liver disease is sufficient proof of failure of one of the synthetic functions of the parenchyma. (4) A normal hippuric acid synthesis test practically excludes impairment of parenchymal function. (5) The thymol turbidity, Takata Ara, Congo red, Colloidal gold, and the cephalin cholesterol flocculation tests are procedures found to give positive results in liver disease. They seem to indicate active damage in the parenchyma. (6) Tests to differentiate extrahepatic obstruction from inflammatory jaundice are on the whole unsatisfactory. This is understandable because cholangitis and consequent parenchymal damage sooner or later supervene in the majority of cases of obstruction to the bile ducts. It is therefore only in the earlier stages that negative reactions to the empirical tests for liver damage or normal responses to the tests for synthetic ability can be expected. Abnormal results in the various tests are valuable confirmatory evidence of the conclusions reached on clinical grounds.

LIVER BIOPSY

Without much doubt one of the greatest advances in the study of liver disease in the last decade has been the development and perfection of the technique of needle biopsies of the liver. There are at present three standard methods of liver biopsy. The first and oldest is surgical biopsy associated with laparotomy, the second, the peritoneoscopic biopsy, and the third, the needle biopsy developed by Roholm and Iversen.[119]

Any liver biopsy provides only a small sample of the total liver substance. A generous surgical liver biopsy may measure about 1 cm. in diameter and represents but about 1/1000 of the total liver substance. A peritoneoscopic biopsy averages 3×3×4 mm. and represents approximately 1/40,000 of the liver. Results of needle biopsy are very variable and range from 1:25,000 to 1:50,000 of the liver volume. It is obvious that such small samples of the liver can permit correct diagnosis of the lesion only when that lesion is diffuse and not focal. But for

tunately, a large proportion of liver lesions are diffuse and, for the most part it is only the diffuse lesions that produce symptoms of liver disease Any form of liver biopsy if properly carried out should permit the diagnosis of viral hepatitis, hemochromatosis amyloid or fatty infiltration biliary cirrhosis diffuse fatty (nutritional) cirrhosis Each method has its advantages and drawbacks

Surgical biopsy is undoubtedly most satisfactory when there are proper indications for performing a laparotomy An operating room and general anesthesia must be used The surfaces of the liver may be inspected and when focal lesions are seen, a biopsy of such a lesion taken It provides an ample amount of material not only for histological sections but for certain histochemical examinations It also permits adequate precautions as to hemostasis Such biopsies, however, are probably only justifiable when the patient s condition warrants an exploratory laparotomy and there are other adequate indications for such a laparotomy During the exploration the liver margin has been frequently retracted for long periods of time with metal instruments Anoxemic damage even acute liver cell necrosis occasional thrombi in the sinusoids hemorrhage and acute polymorphonuclear infiltration may develop under such conditions and evidence of parenchymal damage must be interpreted with wary scepticism [98]

Peritoneoscopic biopsy is free of some of these disadvantages Only local anesthesia need be used In experienced hands it is a relatively safe procedure The liver surfaces can to some extent be inspected and a biopsy or biopsies taken from focal lesions Operating room technique with surgical asepsis must be used and the biopsies can penetrate only 0 3 mm to 0 4 mm into the liver substance Interpretation of apparent fibrosis in such biopsies is very difficult as one may be misled by capsular thickening focal subcapsular scars and the like

The third method that of needle biopsy of the liver has been even more important as regards a greater extension of our knowledge of the pathology of the liver The subject was reviewed by Hoffbauer[6] in 1944 The original studies were carried out by Lucatello[83] in 1895 Various methods have been employed using different sized needles Small bits of tissue were secured by means of suction applied during the advancement of the needle into the liver This method was very successful when used by a group of Danish investigators Roholm and Iversen[119] and Roholm and Krarup[120] They describe a posterior lateral approach and employ a needle with a serrated cutting edge The same technique has been utilized by Dible McMichael and Sherlock[3] in 1943 in a study of the changes in *epidemic and other forms of hepatitis*

Tripoli and Fader[141] in 1941 discussed the use of a new biopsy needle the Silverman Needle for this purpose They advocated an anterior approach entering the liver beneath the costal margin at the lateral edge of the right rectus muscle The Silverman Needle was described in 1938 by Silverman[134] *for use in tumor* biopsy It is of ingenious design and consists essentially of a double needle The outer needle is a 14 gauge hypodermic needle with a bevel tip The inner needle which i split longitudinally down the hub fits inside of the outer needle Before use the two needles are assembled so that the tip of the inner needle is at the beveled tip of the outer one When introduced into the liver the inner split needle cuts a small cylinder approximately 2 mm in width and from *2 to 3 cm* in length of liver tissue which is trapped between its two prong The biopsy is cut from its point of attachment by advancing the outer needle over the inner one and then the two needles are withdrawn simultaneously The procedure is carried

out under local anesthesia, does not need the use of an operating room and if one is careful to anesthetize the parietal peritoneum, causes little discomfort to the patient A lateral approach through the pleura and diaphragm is preferred by many, as it is considered less hazardous if the liver is normal in size

Needle biopsy is therefore a relatively simple and quickly performed procedure It is not however, an entirely safe one Mallory[98] in 1949 reports 2 deaths in 500 biopsies (0 4 per cent) and feels that this is probably an average figure Himsworth[61] in 1947 states (p 137) 'But the procedure (puncture biopsy of the liver) is not without its danger and its limitations *Its main danger is haemorrhage from* the perforation in the liver, and puncture biopsy should never be done on any patient who is either bleeding or whose prothrombin index is subnormal " Hoffbauer[6-] in 1947 further states that it should not be done unless there is an enlarged, *easily palpable liver* For cases with a liver of normal size, a procedure which he calls laparoscopy' should be used This amounts essentially to a needle biopsy in which the biopsy of the liver is watched through a peritoneoscope It has the advantage that the biopsy site can be selected and the danger of the biopsy needle entering the bowel or gallbladder is eliminated

That such needle biopsies have been of tremendous value in increasing our knowledge of liver disease is not debatable It makes possible definite diagnoses in many patients in whom other tests have been inconclusive It has given us a chance to follow the course of non fatal liver disease such as viral hepatitis It has also given an opportunity to judge the efficacy of treatment in such conditions as diffuse fatty (nutritional) cirrhosis As Himsworth[61] states (p 138) it is still *a matter of controversy whether in view of its slight but undeniable risk the pro*cedure is justifiable in clinical practice He concludes however that the advantages of its discriminative use seem to outweigh its dangers This would seem to be pretty much the universal opinion of all authorities on the subject

NEWER CONCEPTS OF NORMAL AND ABNORMAL BLOOD CIRCULATION

(1) **Normal Circulation** —In recent years much fascinating work has been done in relation to the blood circulation of the normal and the pathological liver Marked advances have been made in our understanding of these problems by *using a combined experimental and physiological approach*

Knisely [78] in 1949 reported his studies on the blood flow through the normal frog liver transilluminated in situ by means of fused quartz rods He demonstrated that the hepatic artery and portal vein send branches to the periphery of each liver lobule *The arterioles run along the interlobular portal venule like a* vine on a tree Every liver sinusoid is found to connect with a terminal venule Every sinusoid or almost every one is also connected with an arterial twig from the hepatic artery He found contractile arterio portal anastomoses interconnecting *hepatic arterioles and portal venules He felt that he could demonstrate* an afferent inlet sphincter and an efferent outlet sphincter for each sinusoid He states that the relative composition of the blood supplied to the sinusoids is dependent on the degree of dilation or tonic contraction of the hepatic artery the arterio portal *anastomosis, and the afferent* interlobular portal venule

According to his views, these sphincters cause the liver to store or release blood and in this way affect the circulating blood volume and cardiac output

Similar studies were made by Seneviratne[131] in 1949 on frog, mouse and cat livers using a very similar technique His findings in the three animals were essentially the same as those of Knisely[78] for the frog save that he could not demonstrate the sphincters described by Knisely

He was very much impressed by the readiness with which the circulation of the lobule reacted to various stimuli Under normal conditions he found an irregular intermittent rhythm to the blood flow through the sinusoids Adrenalin led to a marked constriction of all the ramifications of the portal radicals, the sinusoids and the hepatic arterioles A wide variety of stimuli such as trauma burns heat and cold, caused a temporary contraction of the sinusoids There was also considerable effect on this circulation by respiration, a constriction occurring during deep inspiration

Bollman[11] in 1945 reported a study of hepatic blood flow using a device called a ' thermostromuhr which measures blood flow by the cooling effect that it has on a heated capsule placed in contact with the vessel By this means he found that 75 to 80 per cent of the blood to the liver came through the portal vein and 20 to 25 per cent through the hepatic artery This was altered by all the factors that affect the general circulation and after a meal the amount of portal blood flowing through the liver may increase by 200 to 300 per cent

Investigation of the distribution of the portal blood stream has led to some interesting and significant discoveries Although it had been suspected for quite a while that different portions of the liver received blood from different regions of the alimentary tract Copher and Dick[1] in 1928, first demonstrated the correctness of this supposition They injected dye into the splenic or superior mesenteric vein and found that the currents from these vessels kept to the left and right respectively in the portal vein As a result of this the blood from the intestines passes mainly up the right branch of the portal vein to the right lobes while that from the spleen goes to the left lobes This has been confirmed in other animal experiments and is used by Himsworth[61] as an explanation of why in dietary necrosis the left lobe is more affected than the right

(2) **The Circulation in Cirrhosis** —The vascular picture in the liver is markedly altered in cirrhosis One of the first studies of this was done by McIndoe[10] in 1928 By the injection of the portal hepatic venous trees with celloidin he demonstrated that the total vascular bed in cirrhosis is markedly reduced from what it is found to be in the normal liver Kelty Baggenstoss and Butt[74] in 1950 give a good discussion of how this comes about Degeneration and necrosis of the liver cells leads to a collapse of the sinusoids the connective tissue and the reticular framework Regenerating nodules of liver tissue displace the sinusoids and reticulum towards the periphery so that the central veins are found close to the perilobular connective tissue surrounding the regenerated nodule With further injury and further regenerated nodules this process becomes more and more marked The result is thick connective tissue bands between the remaining nodules of parenchymal tissue Although most of the sinusoids are obliterated by this process it is believed that some remain open and through these the portal blood may flow into the central and smallest hepatic veins without coming into contact with liver cells In this way an internal portacaval shunt is developed The blood supply to the regenerated nodules is believed to be mainly arterial the

arterial blood emptying into eccentrically and peripherally placed central and hepatic veins

(3) **Experimental Vascular Lesions** —Experimentally partial and complete obstructions of the hepatic blood vessels have been produced and the results compared with similar lesions observed in man Himsworth,[61] in 1947 gives a good review of this subject

Sudden, complete obstruction of the portal vein in a short time causes death from paralysis of and hemorrhage into the intestines The same result and consequence is seen in man as the result of thrombosis of the portal or mesenteric veins If one of the main branches of the portal vein in the liver is occluded atrophy and fibrosis of the portion of the liver supplied by the obstructed vessel occurs No conspicuous necrosis of liver substance occurs A similar picture occurs in the whole liver if an Eck fistula is produced

In the normal liver a sudden acute occlusion of the hepatic artery results in necrosis of the liver and death of the animal or individual Recently Markowitz[101] has found that although in the dog tying of the hepatic artery causes necrosis of the liver and prompt death within twenty four hours it if receives a large dose of penicillin it lives for five or six days and the liver appears normal If the penicillin is kept up for ten days the dogs survive indefinitely thereafter with no further penicillin The apparent explanation of this phenomenon is *found in the histological examination of the necrotic livers* The portal veins are found to be filled with masses of bacteria This is not true of the livers of those animals that are given penicillin As similar bacteria have not been described in human livers with obstruction to the hepatic artery where the picture seems to be that of bland anemic infarction it would seem possible that this discovery is not entirely applicable to man

Experimental partial obstruction of the hepatic veins results in a central *hemorrhagic necrosis followed by fibrosis around the central veins In Chiari s* syndrome where there is an obliterating endophlebitis of the hepatic veins a similar situation develops and it is also found in right sided cardiac failure The cause of this central hemorrhagic necrosis has long been disputed among pathologists many of whom have explained it on the basis of a high pressure in the inferior vena cava Himsworth[61] points out that if this were the case unless the direction of the portal flow of blood were reversed—of which there is no evidence—*the necrosis should be peripheral in the lobule rather than central* He feels that the only possible explanation for this necrosis is a lack of nutrition or oxygen to the central cells

(4) **Anoxia of the Liver** —Himsworth makes several interesting and worth while points about the relation of anoxia to *liver cell necrosis It has been shown* that if the lobule is deprived of arterial blood necrosis occurs and that the most important function of the arterial blood is to bring oxygen to the liver cells Similarly it has been shown that man or animal exposed to an atmosphere deficient in oxygen develops central necrosis of the liver As has been described above a similar lesion develops when the oxygen supply to the liver is diminished in cardiac failure

Anoxia is however relative to and dependent on the need of the tissue for oxygen If the need of the liver cells for oxygen is increased as it is in hyperthyroidism an oxygen tension in the hepatic blood which is sufficient under normal conditions becomes inadequate and liver changes which range from

centrilobular fatty degeneration or necrosis to massive necrosis develop A similar increased need of oxygen occurs in pregnancy The oxygen tension seems more important than the actual amount of oxygen brought to the tissues

Himsworth[61] feels that in the diseased liver many of the changes found may be consequent to derangements of the circulation With toxic lesions such as that produced by carbon tetrachloride the toxin seems to produce a swelling of the cells on the peripheries of the lobule which compresses the sinusoids and results in an ischemia and necrosis of the central cells In massive hepatic necrosis (acute yellow atrophy) swelling of the periportal liver cells and periportal hemorrhages may prevent normal circulation and contribute to the necrosis Infiltrations of the liver, such as fat compress the sinusoids and throttle sinusoidal circulation As has been pointed out above the distortion of the circulation in cirrhosis decreases the circulation to the remaining liver tissue

Following this line of reasoning he points out that in many abnormal conditions of the liver the lesion which dominates the present state and future course is due not to the direct and specific action of the pathogenic agent which initiates the illness but to a secondary phenomenon consequent upon a nonspecific derangement of the intrahepatic circulation These are secondary rather than primary lesions

(5) **Portal Hypertension** —Portal hypertension and the symptoms it produces result in some of the more serious clinical problems that arise in cirrhosis and chronic hepatitis Any marked degree of portal obstruction will cause portal hypertension As explained above such obstruction occurs in cirrhosis of the liver probably as a result of pressure on the portal blood vessels due to the expanding nodules of regeneration Herrick[59] in 1907 found obstruction to the inflow of portal blood on perfusing cirrhotic livers It has also been demonstrated by direct measurement of the pressure in the portal vein at operation on patients with cirrhosis (Thompson 1940)[149]

As a result of the portal hypertension collateral circulation develops The three most important sites for this collateral circulation are at the junction of the esophagus and stomach the anus and rectum and around the umbilicus As far as the patient is concerned those at the cardio esophageal region are by far the most important as their rupture produces gastrointestinal hemorrhage which is a common cause of death in cirrhosis

The relationship of ascites to portal hypertension is perhaps not quite so clear cut, but most authorities will agree that the hypertension is one of the important factors in its production Other contributing factors in the production of ascites are hypoproteinemia and perhaps the antidiuretic principle found by Ralli *et al*[115] (1945) in the urine of patients with liver disease

In recent years many surgical measures have been tried in attempts to prevent death from rupture of esophageal varices Most of these were summarized by Linton[8] in 1948 The operations tried have been (1) ligation of the splenic artery (2) splenectomy (3) omentopexy (4) ligation of the left coronary and gastric veins (5) transthoracic ligation of the periesophageal veins (6) total gastrectomy (7) esophagogastrectomy (8) injection of the esophageal varices with a sclerosing solution and (9) the creation of a portacaval shunt All but the last have on the whole yielded poor results

The method recommended and used by Linton[8] combines a splenectomy and end to side anastomosis of the splenic and renal veins The left kidney is not

removed or injured It has been used not only in patients where the portal hyper tension is due to intrahepatic portal bed block as in cirrhosis, but also where extrahepatic bed block, such as occurs with portal thrombosis, has been present Following this operation the reduction in portal pressure has been very encourag ing in the extrahepatic group and not quite so dramatic in the intrahepatic group It is still too early to determine the ultimate value of this type of surgery, but to date if a satisfactory shunt has been accomplished, no patients have bled from varices, and the liver function does not seem to have been seriously damaged

Rienhoff[117] (1951) has recently developed a new operation for the treatment of portal hypertension This consists of a ligation of the common hepatic artery distal to the departure of the gastroduodenal artery along with and in conjuction with a ligation of the splenic artery as it arises from the coeliac axis Each of the patients was given both penicillin and streptomycin before and after the opera tion At the time the paper was sent in for publication 6 cases had been success fully operated on in this manner Before publication occurred, 6 more had been done with equal success

It has been done only on individuals with a diagnosis of alcoholic or Laennec's cirrhosis The clinical manifestations of portal hypertension, such as bleeding from esophageal varices and/or ascites have apparently been allayed by this operation The rationale of the operation is that experiments have proven that tying the hepatic artery reduces the portal blood pressure The splenic vein contributes not pressure but blood volume to the portal system Rienhoff, however feels that it is necessary to tie it too, to insure the success of the opera tion He states that he does not presume that this operation will always be suc cessful and much may depend on the stage the disease has reached at the time of operation Its simplicity recommends it and it seems well tolerated by moder ately sick patients

VIRAL HEPATITIS

Introduction

Under this heading the group of diseases associated with the pathological and clinical findings of hepatitis of probable viral etiology will be discussed The first and perhaps most important of these conditions is known as Infectious Hepatitis The second is usually known as Homologous Serum Hepatitis In this second group are included cases of viral hepatitis which have been acci dentally and artificially produced as a result of introducing into the blood or tissues an agent containing human blood serum infected with a virus capable of producing a hepatitis which according to all observers is very similar clinically to ' Infectious Hepatitis and so similar pathologically that the lesions seem iden tical and indistinguishable

Because of this a general discussion of Infectious Hepatitis will first be given followed by a comparison of it with the Homologous Serum Hepatitis and finally by a description of the pathology common to both groups

Infectious Hepatitis

(a) **Nomenclature** —In the past this condition has been referred to as catar rhal jaundice or non-spirochetal infectious jaundice For a period after 1937 it

was referred to as infectious hepatic jaundice At present the approved terminology seems to be 'infectious hepatitis' for the sporadic cases and 'infectious epidemic hepatitis' for those cases which occur during outbreaks

(b) **History** —Infectious hepatitis is certainly not a new disease Its origins are obscured as a result of the fact that until modern times it was not clearly differentiated from other types of infectious jaundice such as Weil's disease and yellow fever Hippocrates knew and wrote about contagious jaundice In the eighth century A D Pope Zacharias and Saint Boniface described an epidemic of jaundice Considerable contagious jaundice occurred during the American Civil War This was also true during the First World War at Gallipoli and the Dardanelles in 1915–1916 Some of these early attacks were undoubtedly Weil s disease, as this disease was not well recognized

From the pathological standpoint Rokitansky[11] in Vienna in 1842 described jaundice due to massive necrosis of the liver This was the picture that has been known for years as 'acute yellow atrophy' of the liver In 1865, Virchow[143] described a benign transient form of jaundice which occurred most commonly in young people He considered this to be the result of a catarrhal inflammation of the biliary system with a mucous plug forming in the papilla of Vater It was from this description that the terminology 'catarrhal jaundice' arose The concept that these two conditions might be manifestations of the same disease gained no credence although it was suggested by Flindt[38] in 1890, Cockayne[19] in 1912 and a group of Scandinavian investigators Lindstedt[81] in 1919 Ehrstrom[30] in 1927 and Wallgren[145] in 1930 An understanding of this problem was not reached until after 1942 when attention was focused on this disease by an outbreak of jaundice of considerable magnitude which occurred in the Army of the United States and in the British Army As a result of this outbreak many investigations from the clinical bacteriological and pathological standpoint have been carried out and have led to a marked clarification of our understanding of the disease

(c) **Etiological Agent** —It is now believed that infectious hepatitis is caused by a specific virus or a specific group of viruses Havens[52–4] in 1945 and 1946 describes a filterable agent, presumably a virus which was resistant to 56° C for at least thirty minutes Although investigation of this virus has been handicapped by the fact that no susceptible animal can be found considerable progress has been made as the result of the use of human volunteers for the purposes of the study By this means it has been found that the virus is present in the stool and serum during the pre icteric and the early icteric stages of the disease It has been transmitted to volunteers by feeding or parenteral inoculation of these materials Similar studies of the infectivity of urine and nasal washings have proven contradictory

From two widely separated outbreaks of infectious hepatitis Henle *et al*[66] in 1950 obtained two strains of viruses one called Akiba and the other the NL Strain These were obtained from the serum and stools of patients They were grown in tissue cultures of rabbit liver cells in roller tubes and in minced chick embryos in the Simms-Sanders medium This was followed by the passage of the virus in the amniotic cavity of the chick Cultures of both of these strains of viruses induced mild hepatitis without jaundice in the majority of the volunteers tested Drake *et al*[4] in 1950 continued this work by comparing the disease produced by the cultured virus with that produced by the infection with the

removed or injured It has been used not only in patients where the portal hyper tension is due to intrahepatic portal bed block as in cirrhosis but also where extrahepatic bed block, such as occurs with portal thrombosis has been present Following this operation the reduction in portal pressure has been very encouraging in the extrahepatic group and not quite so dramatic in the intrahepatic group It is still too early to determine the ultimate value of this type of surgery, but to date if a satisfactory shunt has been accomplished, no patients have bled from varices, and the liver function does not seem to have been seriously damaged

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Infectious Hepatitis

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cedures used in immunization against or in therapeusis of conditions unrelated to the hepatitis

Probably the largest numbers of patients fall into the group that have such a hepatitis following blood or plasma transfusions One of the first reports of this condition was given by Rappaport[116] (1945) He described hepatitis occurring in 32 patients evacuated from overseas who developed jaundice nine to eighteen weeks after a blood or a plasma transfusion Paul and Havens *et al*[11] (1945) Neefe *et al*[107] (1945), Havens[58] (1946), and Stokes[137] (1951) have isolated the virus and carried on transmission experiments using human volunteers Stokes summarized this work and compared the virus of serum hepatitis with that of infectious hepatitis He found that the incubation period in infectious hepatitis was twenty to forty days and in serum hepatitis sixty to one hundred twenty days Secondary cases were common in infectious hepatitis and uncommon in serum hepatitis The virus could be isolated from the feces and blood in infectious hepatitis and from only the blood in serum hepatitis Infectious hepatitis was uncommon after forty years of age, whereas serum hepatitis was more common after forty years of age Oral administration of infected serum produced infectious hepatitis but failed to produce serum hepatitis Both viruses showed evidence of homologous immunity but no evidence of heterologous immunity

Similar serum hepatitis has been apparently produced in a variety of other ways Rosenthal *et al*[1 3] in 1950 reported serum hepatitis produced by plasma despite irradiation of this plasma with ultraviolet rays Thrombin used for hemostasis in surgery was incriminated by Lesses and Hamolsky[79] in 1951 They noted that no viricidal techniques were used in the preparation of the thrombin It has also followed the use of convalescent serum for measles and mumps, Beeson *et al*[5] 1944, and the immunization against sand fly and yellow fever, Runyan *et al*[1 6] 1950 It has also been transmitted by syringes and syringe needles which have not been properly sterilized, in the administration of arsphenamine for syphilis and the administration of insulin in the treatment of diabetes (Capps *et al*[14] 1948 Sherwood[133] 1950 Droller) It has been reported to follow even tattooing with a common needle (Roberts and Still[118] 1950) (Smith[13] 1950)

The hepatitis following vaccination against yellow fever (Findlay[35] 1944) and the hepatitis seen in arsenic therapy for syphilis have been worked up quite extensively

Hepatitis Following Vaccination Against Yellow Fever —This was seen in both American and British troops in the Second World War as all troops going to areas where yellow fever was endemic were so immunized The vaccine used contained attenuated yellow fever virus suspended in human serum From 1 to 10 per cent of the batches used apparently contained the virus of serum hepatitis Almost 50 000 cases of serum hepatitis followed the use of this vaccine The incubation period varied from three and one half to thirty four weeks The hepatitis was rarely fatal the mortality being between 1 45 and 2 per 1 000 cases The disease was transmitted to volunteers by Oliphant Gillian and Larson[110] in 1943 and by Oliphant[111] in 1944 They produced hepatitis both by using a so called icterogenic strain of vaccine and by inoculating pooled serum from human cas s with hepatitis which had followed the use of the vaccine According to Badger[3] (1944) jaundice ceased following the vaccination when use of human serum in its manufacture was discontinued All authorities agree that the clinical

uncultured virus from the same source They felt that the disease produced was the same

Henle *et al*[67] in 1950 and Stokes[137] in 1951 discussed a skin test antigen for infectious hepatitis obtained from the amniotic fluid of the chick embryos infected with the virus of infectious hepatitis As a result of about 5,000 such skin tests they felt that they had obtained specifically positive skin reactions in all individuals with past histories of spontaneous or induced infectious hepatitis In contrast to these findings the incidence of positive tests among cases of post serum hepatitis was no greater than that found in an adult group chosen at random

(d) **Epidemiology** —In 1946 Hardy and Feemster[48] discussed the epidemiology of infectious hepatitis All ages seem susceptible, but the disease is most common between the ages of six and forty years Both sexes seem to be equally susceptible although males are more apt to be massed together under conditions favoring epidemics than are females The geographic distribution seemed to be world wide It is very difficult to get any worthwhile concept of the incidence Sporadic cases are always present Epidemics occur from time to time in the Army, Navy in camps and institutions Only in Finland and Switzerland is it a reportable disease and in these two countries 65 000 cases were reported in 1943 There seems to be no striking seasonal incidence

Studies on immunity have been hampered because of the difficulties in identifying the specific virus causing the infection Much confusion has arisen due to the fact that the infectious hepatitis and serum hepatitis seem to be caused by similar but antigenically different viruses The general impression has been that reinfection by the same virus is very exceptional Evidence corroborating this has been published by Stokes[137] in 1951 Volunteers infected with the infectious hepatitis virus had hepatitis following the first dose of the virus but did not have it following a second dose of the same virus The same volunteers again had hepatitis with jaundice when given the serum hepatitis virus but showed no hepatitis to a second dose of this same virus The findings were similar when the first two doses consisted of serum hepatitis virus and the next two were of the infectious hepatitis virus

In experimental infections Havens[54] (1946) found the incubation period to be between sixteen and thirty days This was unaffected by the size of the dose given Studies of epidemics would indicate that the incubation period of the naturally acquired disease is quite variable It has been suggested that this may be due to a difference in virulence of the virus

The methods by which the disease is transmitted are still somewhat in dispute As the virus is known to be present in the feces fecal contamination of water and food would seem to be the most obvious method of spread of the disease Neefe and Stokes[106] (1945) described an epidemic in a camp which was apparently due to fecal contamination of water Hardy and Feemster[48] (1946) felt that the cases in Massachusetts could be best explained due to droplet infection from coughing Attempts to demonstrate the virus in nasal pharyngeal washings however have not been very successful

Serum Homologous Jaundice

As has been mentioned above this terminology is used for cases of viral hepatitis which have been accidentally and artificially produced resulting from pro

cedures used in immunization against or in therapeusis of conditions unrelated to the hepatitis

Probably the largest numbers of patients fall into the group that have such a hepatitis following blood or plasma transfusions One of the first reports of this condition was given by Rappaport[116] (1945) He described hepatitis occurring in 32 patients evacuated from overseas, who developed jaundice nine to eighteen weeks after a blood or a plasma transfusion Paul and Havens *et al*[112] (1945) Neefe *et al*[107] (1945), Havens[53] (1946), and Stokes[137] (1951) have isolated the virus and carried on transmission experiments using human volunteers Stokes summarized this work and compared the virus of serum hepatitis with that of infectious hepatitis He found that the incubation period in infectious hepatitis was twenty to forty days and in serum hepatitis sixty to one hundred twenty days Secondary cases were common in infectious hepatitis and uncommon in serum hepatitis The virus could be isolated from the feces and blood in infectious hepatitis and from only the blood in serum hepatitis Infectious hepatitis was uncommon after forty years of age, whereas serum hepatitis was more common after forty years of age Oral administration of infected serum produced infectious hepatitis but failed to produce serum hepatitis Both viruses showed evidence of homologous immunity, but no evidence of heterologous immunity

Similar serum hepatitis has been apparently produced in a variety of other ways Rosenthal *et al*[123] in 1950 reported serum hepatitis produced by plasma despite irradiation of this plasma with ultraviolet rays Thrombin used for hemostasis in surgery was incriminated by Lesses and Hamolsky[9] in 1951 They noted that no viricidal techniques were used in the preparation of the thrombin It has also followed the use of convalescent serum for measles and mumps Beeson *et al* 1944 and the immunization against sand fly and yellow fever, Runyan *et al*[124] 1950 It has also been transmitted by syringes and syringe needles, which have not been properly sterilized in the administration of arsphenamine for syphilis and the administration of insulin in the treatment of diabetes (Capps *et al*[14] 1948 Sherwood[133] 1950 Droller) It has been reported to follow even tattooing with a common needle (Roberts and Still[118] 1950) (Smith[14] 1950)

The hepatitis following vaccination against yellow fever (Findlay[3] 1944) and the hepatitis seen in arsenic therapy for syphilis have been worked up quite extensively

Hepatitis Following Vaccination Against Yellow Fever — This was seen in both American and British troops in the Second World War as all troops going to areas where yellow fever was endemic were so immunized The vaccine used contained attenuated yellow fever virus suspended in human serum From 1 to 10 per cent of the batches used apparently contained the virus of serum hepatitis Almost 50 000 cases of serum hepatitis followed the use of this vaccine The incubation period varied from three and one half to thirty four weeks The hepatitis was rarely fatal the mortality being between 1 45 and 2 per 1 000 cases The disease was transmitted to volunteers by Oliphant Gilliam and Larson[110] in 1943 and by Oliphant[111] in 1944 They produced hepatitis both by using a so called icterogenic strain of vaccine and by inoculating pooled serum from human cases with hepatitis which had followed the use of the vaccine According to Badger[3] (1944) jaundice ceased following the vaccination when use of human serum in its manufacture was discontinued All authorities agree that the clinical

picture and pathology found in these cases was that of viral hepatitis rather than that of yellow fever

"Arseno-Therapy" Hepatitis —This was first reported by Ruge[12] (1925) who found that the jaundice occurring in the syphilitics in the German Navy increased proportional to an increase in non spirochetal jaundice occurring in the general population Similarly Beattie and Marshall[4] (1944) found a rise in jaundice in syphilitics corresponding with a rise in epidemic hepatitis Roholm and Iversen[119] (1939) and Dible, McMichael and Sherlock[23] (1943) did needle biopsies on these livers and found the pathological picture similar to that of infectious hepatitis The incidence of this jaundice was substantially reduced by the introduction of aseptic technique (Salaman [1 7] 1944) MacCallum[86] (1944) transmitted this hepatitis to volunteers by the inoculation of serum from two cases who had developed jaundice at the nineteenth week of treatment Fecal suspensions by mouth did not produce the hepatitis The incubation period was found to be between forty two and eighty days

One of the unexplained mysteries of all these groups of serum hepatitis is why certain sera contain viruses when such care is taken to use blood only from those who give no history or evidence of hepatitis It has been suggested that healthy carriers may exist but no actual proof of this has as yet been reported

Pathology

First, the pathology of *Fatal Viral Hepatitis* will be discussed and secondly that of the much more common form of *Non Fatal Hepatitis* will be described The mortality in most epidemics has been only 0 2 to 0 4 per cent

Fatal Viral Hepatitis —Most cases of Fatal Hepatitis fall into one of two groups The first of these is sub acute and was described by Lucke[84] in 1944 and the second is the fulminant form and was described by Lucke and T B Mallory[85] in 1946 Although the clinical and pathological manifestations of the two types are quite different there are enough points of similarity and enough transitional forms are found to make it obvious that the disease is one and the same process

(a) **Subacute Fatal Hepatitis** —This description is based on the publication of *Lucke[84] in 1944 in which he describes an outbreak of hepatitis in the American* Army during 1942 and 1943 He described 125 fatal cases all of which fell into this subacute group The clinical picture was essentially the following The duration of sickness from onset to death was from twenty to fifty days in 59 per cent of these cases In none of them was it less than ten days and in a few one hundred days or more

Clinically he described three phases or stages a pre icteric stage an intermediate stage and a final stage The duration of the pre icteric stage in the majority of cases was seven days or less The main symptoms in this phase were anorexia nausea dark urine abdominal distress vomiting malaise and weakness The duration of the intermediate stage was usually twenty six days or less The symptomatology was very similar to the preceding stage On physical examination there was jaundice and usually a large tender liver The majority showed no signs or symptoms to lead one to believe that their course was not to be the usual benign one Many were allowed up and around the ward In the majority of patients the final stage lasted ten days or less Usually this was ushered in by a sudden dramatic change for the worse The patients developed persistent

vomiting ascites and nervous symptoms such as restlessness delirium, and coma

HEPATIC PATHOLOGY —The liver showed by far the most significant pathology The lesion was essentially that which has been described for many years as sub acute yellow or red atrophy The livers were small, usually weighing between 1 000 and 1 400 grams and sometimes as little as 600 grams They were grossly deformed, showing large, depressed, atrophic, red areas and large yellow nodules of regeneration Histologically in the atrophic areas there was a complete dis appearance of liver cells The lobular structure could still be made out as it was outlined by large numbers of small proliferating bile ducts The sinusoids were preserved and usually engorged with red cells This accounted for the gross red color The reticular framework was not destroyed Its meshes were narrowed, the fibers shortened and thickened The surviving connective tissue showed little or no collagen formation There was invariably an inflammatory reaction pre dominantly in the portal areas In the early stages this consisted of about equal numbers of polymorphonuclear leukocytes, lymphocytes, plasma cells, and macro phages In the later stages the polymorphonuclear leukocytes decreased in num ber Another quite characteristic change was an endophlebitis involving the efferent blood vessels This was most prominent in the central lobular and sub lobular veins The wall of the vein was thickened and appeared hyalinized Beneath the endothelial lining was an exudate consisting of polymorphonuclear leukocytes, lymphocytes, plasma cells, and pigmented macrophages The picture in the nodules of regeneration was quite different There was marked evidence of hypertrophy and multiplication of the cells which had escaped destruction Binucleate and multinucleate cells were common The lobular architecture was usually atypical The lobules varied in size being usually larger than normal and there was no constant relationship between the central vein and the periphery of the lobule as shown by the portal areas The liver cords were broad and irreg ular and the bile canaliculi were dilated The bile canaliculi often showed bile stasis as evidenced by inspissated masses of bile in their lumens

EXTRA HEPATIC PATHOLOGY —The extra hepatic pathology was not too note worthy Sometimes a small plug of inspissated bile was found in the common bile duct or the ampulla The lymph nodes particularly the regional ones often showed a lymphoid hyperplasia In about 60 per cent there was ascites usually of 2 liters or more The weight of the spleen was commonly from 200 to 400 grams It showed essentially, congestion and hyperplasia In rare cases the spleen weighed as much as 5 or 6 hundred grams This occurred only in those individuals with a prolonged clinical duration of the disease The probable ex planation of this was on the basis of a portal hypertension Involvement of the kidneys varied Frequently there was a moderately severe cholemic nephrosis A phlegmonous enteritis was found in about 15 per cent of this subacute group It occurred only when ascites was present and was predominantly in the ileo cecal region Grossly the affected bowel was boggy and edematous The mucosa was usually unbroken but sometimes showed focal areas of hemorrhage Histologi cally in addition to the edema infiltration with polymorphonuclear leukocytes or macrophages was found With a Gram stain Gram negative bacilli and Gram positive rods could be found in this edematous tissue This enteritis was probably best explained as a terminal infection of the edematous bowel

In spite of the prominent clinical signs of neurological involvement the pathol ogy of the brain was not striking Sometimes a slight lymphocytic infiltration of

the meninges and a moderate perivascular cuffing by lymphocytes around the small blood vessels in the basal ganglia were found. This involvement did not suggest a direct virus infection of the central nervous system.

Many of this series of cases must be considered as homologous serum hepatitis rather than infectious epidemic hepatitis as they followed vaccination against Yellow Fever.

(b) **The Fulminant Form of Fatal Epidemic Hepatitis.**—In 1946 Lucke and T. B. Mallory[80] described 178 cases that fell into this group. The clinical course of these cases was much shorter than that seen in the preceding subacute group. Fifty three per cent died in less than ten days. Many died in three to four days and a few even before the onset of jaundice. Forty three per cent of these individuals had a history of severe trauma or burns within four months of the onset of the hepatitis. Inasmuch as these were all military personnel it is probably true that the majority of them were given blood transfusions at the time of the trauma or burn. This is of importance because it suggests that this 43 per cent may represent *homologous serum jaundice rather than epidemic hepatitis.* Clinically the same three phases described in the subacute group, could be recognized. The pre icteric phase usually lasted only two or three days. Frequently the terminal phase was only one to four days in duration. Sometimes the intermediate phase was so brief that it could not be recognized. The clinical symptoms could be divided roughly into two types. The first of these was called the infectious type and showed itself as fever, chilliness, shaking chills, malaise, and generalized aches and pains. In the second, or gastrointestinal type, the predominant symptoms were anorexia, vomiting, epigastric discomfort, or pain. Sometimes but rather rarely the patient showed combinations of these two types of symptoms. Rarely the terminal phase was ushered in by severe cerebral symptoms. As in the subacute fatal hepatitis, prodromal symptoms did not indicate that the course was going to be fulminant. The final stage in both the subacute and fulminant group was very similar. Jaundice often deepened rapidly, vomiting became severe, and there was often evidence of bleeding from the gastrointestinal tract mucosa. Terminally they often showed signs of shock with pulmonary edema and bronchopneumonia.

PATHOLOGIC ANATOMY.—The most striking and important lesions were found in the liver. Grossly the reduction in size of the liver was usually moderate. The average weight was from 1 000 to 1 400 grams. Only rarely were 600 to 800 gram livers found. The surface of the liver was smooth or finely wrinkled. The large nodules seen in the subacute form were never seen. Small subcapsular areas of hemorrhage were common. These livers were very soft and flabby in consistence. Sometimes this was so marked that they more or less collapsed on the sectioning table. The color of the liver tissue varied and was not distinctive. It could be red, purple, or brown. On sectioning the cut surface not infrequently presented a nutmeg pattern, suggesting chronic passive congestion or central hemorrhagic necrosis.

Microscopically there were two predominant processes. These consisted of (1) a marked destruction of liver cells and (2) a marked inflammatory reaction. The destruction of liver cells was usually uniform throughout the liver, although in rare cases the left lobe of the liver was involved more extensively. The liver cell destruction would involve either the whole lobule leaving no surviving cells or a narrow rim of such cells would be found on the periphery. Characteristically

the necrotic cells were removed rapidly Only rarely were necrotic unremoved cells found Usually the surviving cells showed no degenerative changes The reticulum and sinusoids persisted Very frequently the sinusoids were intensely engorged

Inflammatory Reaction —Cellular infiltration was more marked than that seen in the subacute form It occurred principally on the periphery of the lobules in the portal stroma and at the interlobular boundary As a result of this, the peripheries of the lobules were outlined and prominent The cells of the infiltrate were chiefly mononuclear They consisted of histiocytes plasma cells and lymphocytes Neutrophils and eosinophils were always found but they were relatively small in number Macrophages commonly contained lipofuscin This probably came from the breakdown of the liver cells There was no evidence of intra nuclear or cytoplasmic inclusion bodies such as are found in other types of virus infection The endophlebitis described in the subacute form was rarely found in this fulminant form Also there was no noteworthy evidence of regeneration of liver cells Even in the most fulminant cases however, the small bile ducts on the periphery of the lobules showed evidence of proliferation The lesions de scribed were those found in patients surviving less than ten days About one third of the patients surviving ten to nineteen days showed identical pathology The remaining cases in this period showed intermediate stages between the ful minant and subacute hepatitis

Extrahepatic Pathology —*Ascites* —The majority of these cases showed ascites Its frequency increased with the duration of the disease and the larger the liver found the more apt was it to be present

The spleens showed moderate enlargement due to hyperplasia of the component cells and congestion The degree of the enlargement was not as great as in the subacute cases

The kidneys were usually somewhat enlarged They could be dark due to congestion or pale They were never deeply stained with bile and did not show the severe bile nephrosis seen in the subacute cases With fat stains much fat could be seen in the proximal convoluted tubules With a polarizing microscope this fat was found to be neutral The fat seemed more prominent in those cases of shorter duration and with more fulminant disease As there was little or no evidence of epithelial degeneration of the tubules the fat seemed best to be con sidered as a storage phenomenon rather than as degenerative fat

The brains for the most part were grossly negative Frequently histologically a nonspecific encephalopathy could be found In the most fulminant of the cases the ganglion cells could be seen to be swollen the nuclei distorted and the chro matin granules dispersed and scattered

The Mechanism of the Jaundice —In the subacute cases the mechanism of the jaundice would seem to be primarily mechanical Mechanical obstruction was caused by thrombi of inspissated bile in the interlobular bile canaliculi In the fulminant cases however there was no evidence of bile stasis Here the most obvious explanation of the jaundice was that there were insufficient surviving liver cells to care for the metabolism of the blood pigments

Hepato Renal Syndrome —The strict interpretation of this syndrome is that the renal damage must be consequent to the disease of the liver and that the damage must be so severe as to lead to a uremic death If this strict interpreta tion is followed neither the subacute nor the fulminant cases could be considered

to show the syndrome Some azotemia occurred, but the deaths were primarily cholemic rather than uremic

Non-Fatal Epidemic Hepatitis —As is well known, epidemic hepatitis is essentially and characteristically a benign disease, 99 6 per cent of cases ending in complete recovery It is, therefore, obvious that in order to learn the pathology and follow the course, liver biopsies are necessary This discussion is for the most part based on the publication of T B Mallory[97] in 1947 and on the more detailed study by Horan, Jolliffe and Mallory,[86] as yet unpublished The material discussed by these authors consists of 160 biopsies from 137 cases of non fatal disease They divide their material into two main stages (1) an acute stage and (2), a post icteric stage



FIG 24 —*Benign Viral Hepatitis H matoxylin Eosin* × 20 Periportal and intralobular inflammation. Lobular disarray

(a) **Acute Stage** —There were 54 liver biopsies to represent this stage It is subdivided into

THE ICTERIC CASES —In the icteric stage six characteristic features were found in each biopsy The degree of involvement and severity of each of these features varied markedly from case to case The six features were the following

(1) *1 Periportal Inflammatory Infiltration* —This was predominantly mononuclear and most commonly consisted of histiocytes and lymphocytes Plasma cells were also usually present and there was always an occasional neutrophil or eosinophil to be found Frequently the inflammatory infiltration spread from the portal area to involve the liver cells on the periphery of the lobule No degenerative changes or necroses of these inflammatory cells were seen (Figs 24 and 25)

(2) *In Intralobular Inflammation* - There was a swelling and a hyperchromatism of the reticulo endothelial cells making them conspicuous Often they could

be seen to contain bile pigment and fat Also there were many focal collections of histiocytes lying either in the space of Desse or in the sinusoidal lumens Frequently these histiocytes were clumped around necrotic liver cells

(3) *Lobular Disarray* —Shrinkage or swelling of individual liver cells led to an irregularity in the width of the hepatic columns and frequently caused a break in their continuity Because of this the normal radial arrangement of the hepatic cords was obscured With silver stains the reticular pattern could be seen to be distorted and there were foci of condensation of the reticulum

(4) *Degeneration and Necrosis of Liver Cells* —A quite characteristic form of degeneration and necrosis of individual single liver cells was described In the early stages the cytoplasm became homogeneous and intensely eosinophilic The nucleus was at first pyknotic, then became fragmented and later disappeared

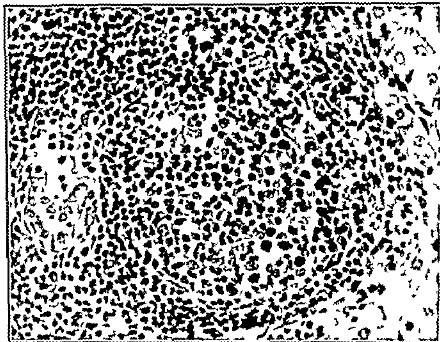


FIG 25 —*Benign Viral Hepatitis Hematoxylin Eosin* × 300 Portal area to show character of inflammatory infiltrate Histiocytes lymphocytes and polymorphonuclear leukocytes can be seen

In the end they became hyaline spherical bodies which were extruded from the cell column into the space of Desse or the lumen of the sinusoids (Fig 26) They resemble somewhat the so called Councilman s bodies of yellow fever Foci of histiocytes tend to form around these necrotic cells This type of necrosis has also been described previously by Roholm and Iversen[119] (1939) Dible and associates[22] and Axenfeld and Brass[2] (1942) all of whom saw it in needle biopsies This type of change could affect individual liver cells anywhere in the lobule The autolytic central necrosis described in the fatal fulminant form was never seen in these biopsies from the benign disease Another less frequent type of hepatic cell degeneration was also found in these biopsies The cells would swell to 4 or 5 times their normal diameter as a result of ballooning of their cytoplasm The

cytoplasm was described as pale with widely separated fine granules No fat was found in these cells with fat stains Their nuclei were sometimes atypical, and many of these cells were multinucleated This type of change seemed to be restricted to the centers of the lobules

(5) *Regeneration* —In the active stage numerous mitotic figures were found in the liver cells These seemed entirely out of proportion to the necrosis of tissue Not infrequently multipolar mitoses were seen (Fig 27) This perhaps accounted for the multinucleated cells Although elongation and tortuosity of the small bile ducts indicated proliferation bile duct epithelial mitoses were considerably rarer (Fig 28)

(6) *Biliary Stasis* —When icterus was present bile stasis was consistently found This was usually restricted to the centers of the lobules Accumulation of bile droplets in the liver cells was also seen

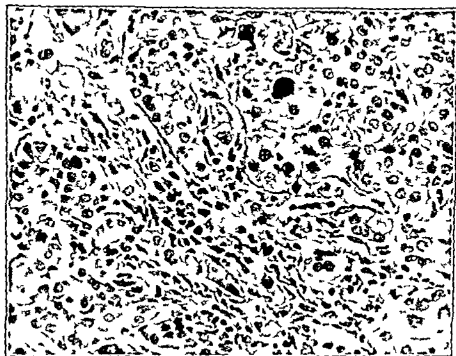


Fig 26 —*Benign Viral Hepatitis Hematoxylin Eosin* × 400 Hyaline body in sinusoids is end result of hyaline necrosis Intralobular inflammation and lobular disarray also present

The Pre icteric Cases —In 7 cases jaundice occurred one to seven days after the biopsy was taken These biopsies were taken on the fifth to the fourteenth day of illness In 6 the liver changes were similar to those described in the icteric cases except that no bile stasis was found In the remaining one bile stasis was found but jaundice occurred twenty four hours after biopsy was taken

Sub icteric Cases —In 5 such cases changes were similar to those in the pre icteric group No bile stasis was found

Non icteric Cases — In 18 individuals hepatitis was clinically suspected although no clinical or chemical evidence of bilirubin retention was found In 5 of these changes similar to those in the icteric group were seen although the bile

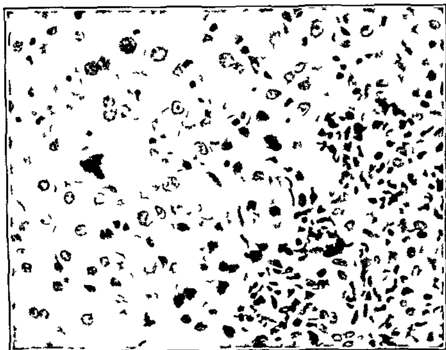


Fig 27 —*Benign Viral Hepatitis Hematoxylin Eosin* × 400 Regeneration as evidenced by a multiple mitosis and binucleate liver cells

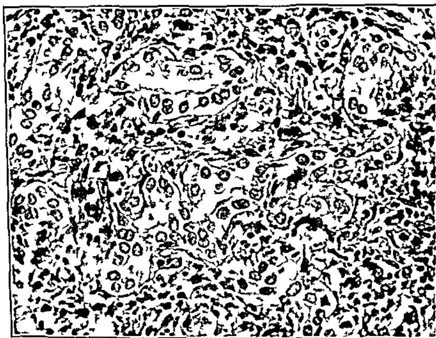


Fig 28 —*Benign Viral Hepatitis Hematoxylin Eosin* × 400 Portal area to show bile duct proliferation and inflammatory infiltrate

stasis was minimal In another 5 the changes were considered as equivocal, as one or more of the six criteria described were absent In 8, the liver biopsies were essentially normal in appearance

(b) Post icteric Stage —It was obvious from clinical and laboratory findings that the disease did not terminate coincidentally with the jaundice This stage was subdivided into five groups

Normal Recovery Group —Of 20 patients in this group 8 had positive biopsies 5 doubtful, and 7 negative The most common finding in the positive ones was the periportal inflammation Inflammatory foci and hyaline necrosis were also frequent There seemed to be an increase of multinucleated cells and a new phenomenon of fat vacuoles in the liver cells was found One case showed slight bile stasis The findings of lobular disarray and frequent mitoses had to a large extent disappeared It became evident that the rate of recovery varied widely even in normal convalescence Two cases had cleared on the thirty second day, 4 cases between the forty fifth and fifty third day On the other hand in 1 case eighty three days after onset, the biopsy showed moderately severe findings

Recrudescences —Recrudescences were the most obvious reason for delayed recovery They commonly occurred from the thirtieth to the fiftieth day of the disease Often these recrudescences were as severe or even severer than the original attack Biopsies taken during them showed changes similar to equivalent times in the initial attack

Recurrent Attacks —Even after prolonged intervals of health recurrent attacks were by no means rare Biopsies were taken on the third, forty eighth and ninety seventh days after the onset of recurrences These recurrences had occurred three hundred three hundred and sixty, and three hundred and ninety days after the original attack The findings in the first of these biopsies were indistinguishable from those seen in the acute stage The second biopsy was normal and the third equivocal No evidence was found in these biopsies of permanent damage from the first initial attack Recent immunological studies suggest that these recurrences might represent an infection with the virus of homologous serum hepatitis

Delayed Recovery —In 40 cases evidence of delayed recovery was given by persistence of the symptoms signs or abnormal laboratory findings The biopsies were taken in a range between one hundred to five hundred days after onset of the disease In 15 of these the liver was found to be normal In 10 the changes were minimal but in 15 there were definite findings of hepatitis In these last 15 periportal and intralobular inflammation was present as was also hyaline necrosis No bile stasis or lobular disarray was found Mitoses were rare

Chronic Non icteric Group —This was not too satisfactory a group as it was composed of 10 clinically debatable cases In only one was there evidence of histologically active hepatitis and this was the only one in the group to show consistently abnormal laboratory findings

FATTY INFILTRATION OF THE LIVER

Of the infiltrative processes that occur in the liver by far the most important is the infiltration by fat This importance depends on the discovery that prolonged extensive fatty infiltration of the liver results eventually in a cirrhosis of the liver of the type which is variously called alcoholic Laennec s or portal cirrhosis and that it is the fat that causes the cirrhotic process

Although it had been noted by a group of physiologists as early as 1932 that after pancreatectomy in dogs the liver became large and fatty, it was not until 1938 that attention was focused on this problem by Chaikoff, Connor and Biskind [16] They pointed out that if these depancreatized dogs were kept alive with the necessary amounts of insulin and given an adequate diet they not only developed fatty livers but in two to five years developed a definite cirrhosis of the liver Later in the same year Connor [0] pointed out that in man fatty infiltration followed by cirrhosis was seen both in chronic alcoholism and diabetes

Meanwhile it was discovered by Best[7] in 1933 that if depancreatized dogs with fatty livers were given choline the fat disappeared from the livers In 1943 Best *et al*,[8] demonstrated that simple choline deficiency in normal animals rapidly produced fatty infiltration of the liver even if the diet contained no fat These findings led to a large number of studies on the relation of fat and cirrhosis to diet As a result of these studies a new terminology for substances influencing the amount of fat occurring in the liver has been developed Definitions of these terms as given by Best[9] in 1944 follow so that the literature can be better understood (p 15)

(1) *Lipotropic* —A term applied to any component natural or synthetic, of a diet which prevents the accumulation or accelerates the removal of lipids from the liver

(2) *Antilipotropic* —A term applied to diets or substances which counteract the action of the lipotropic factors

(3) *Hypolipotropic* —A term applied to diets containing less than the physiological amount of the lipotropic factors

(4) *Alipotropic* —A term applied to diets or substances which exert no lipotropic action

(5) *Lipotropes* —The lipotropic factors

(6) *Lipotropism* —The lipotropic effect

As the result of the intensive study done on fatty infiltration of the liver in the past decade we now know that this fatty infiltration can be produced not only by diets rich in fats but by diets deficient in lipotropic factors Lecithin was found to be lipotropic and then choline was demonstrated as the active principle of lecithin Other substances such as casein egg white and beef muscle were found to be actively lipotropic In testing the amino acids methionine was the only one found to be lipotropic du Vigneaud[26-8] in 1939 1940, and 1941 and his coworkers demonstrated that the relationship between methionine and choline was that the methyl group of methionine was used to synthesize choline As Himsworth[61] pointed out, these considerations explain why adequate supplements of choline to a high fat diet prevent fatty infiltration of the liver whereas this fatty infiltration can develop in animals subsisting on a low fat diet They also explain the action of high protein diets in preventing the lesion

Certain substances the best known of which is cystine seem actually to promote fatty infiltration of the liver The probable explanation of this is that cystine has an antilipotropic action and counteracts the lipotropic action of methionine Certain vitamins also influence fatty infiltration Fatty livers do not develop on a diet deficient in choline unless it contains methionine Inositol (Best [10] 1946) and pyridoxine (Gyorgy [4] 1944) seem to have a lipotropic action Riboflavin (Vint [14] 1937) deficiency is claimed to cause marked fatty infiltration

Fatty infiltration in both the experimental and human lesions shows itself grossly as an enlarged smooth liver, yellow in color, and greasy on section Hartroft et al [49–51] in a series of three papers (1949 1950, and 1951), have made some very interesting histological observations about the development, progress and eventual transformation into cirrhosis of the experimental lesions developing in rats on a diet deficient in choline

The lesions in the rat liver were followed by sacrificing the rats at frequent intervals during the development of the lesions Sections of these livers were stained with routine stains and fat stains Small droplets of fat appeared in the liver cells in the centers of the lobules within one day of starting on the diet Within seven to ten days every liver cell contained a single large fat vacuole which distended the cell in which it was contained This occurred as the result of the fusion of the small fat droplets

As a result of the continued distension of these cells by fat the septa formed by the limiting membranes of adjacent cells ruptured, and the contained fat coalesced to form large fat cysts These sacs with unilocular lumina distended with fat were called ' fat cysts or "lipodiasemata" by Hartroft The walls of these cysts consist of a continuous single layer of ' dedifferentiated' liver cells This process occurred several weeks after the animals had been started on the diet Evidence was seen indicating that the blood flow to the cells in the centers of the lobules was reduced as a result of compression of the sinusoids by the swollen fatty cells

Sections of livers from rats who had been on the diet approximately three months showed evidence of atrophic changes in the fat cysts Associated with this the fibrous tissue from the centers of the lobules condensed around the degenerating cysts This eventually formed fibrous trabeculae around the central veins and in time these extended from one branch of the central vein to another At the same time there was also evidence of nodules of regeneration forming in the periportal tissue

If choline is added to the diet of rats after one week on choline deficiency in twenty four to forty eight hours the fat completely disappears from the liver cells at the periphery of the lobule and two to four days later no fat can be demonstrated in any liver cells It is worth noting that the fat first appears in the centers of the lobules and it is from this zone that it is last to disappear If the stage has been reached where fatty cysts are present before choline is added to the diet it takes a considerably longer period of time for this fat in the cysts to be mobilized and absorbed

Study of the future course of the fat cysts demonstrated that these cysts can rupture either into a bile canaliculus or hepatic sinusoid With fat stains Hartroft demonstrated droplets of fat in the canaliculi and bile ducts Rupture into sinusoids with hemorrhage into the collapsed cysts could also be shown As evidence of rupture into the vascular system small fat emboli were demonstrated in the capillaries of the heart lung and renal glomerulus

Hartfroft feels that the fibrosis in experimental dietary cirrhosis represents a condensation rather than a proliferation of the pre existing stroma In human alcoholic patients with fatty livers he found similar fat cysts and small fat emboli in the glomerular loops of the kidney

In these studies the fat stains on frozen sections were by far the most revealing In routine paraffin sections however it was possible to identify the fatty cysts by the presence of several nuclei in the cyst wall This is easier in shrunken cysts

because, due to condensation more nuclei are more apt to be included in the section

CEROID

Considerable interest has been aroused by a substance, probably a pigment called ceroid which has been found in the livers of rats on diets which produce fatty infiltration and the diffuse hepatic fibrosis of Himsworth[61] It appears in unstained sections as peculiar light greenish globules of variable size and shape mainly enmeshed in the periportal connective tissue It is acid fast in stains used for the tubercle bacillus stains with Sudan IV, and is fluorescent but not soluble in fat solvents It is not present in experimental lesions of the same type produced in dogs and there is considerable doubt as to whether it ever occurs in human livers According to Himsworth[61] it is to be regarded as an incidental result of the diets used in producing fatty infiltration and cirrhosis rather than as an essential factor in the production of liver injury

In the conference on liver injury in 1944 Gyorgy[44] expressed the opinion that the ceroid in these experimental rat livers was the result of the cod liver oil in the diets Endicott[31] produced ceroid in normal animals by the injection of cod liver oil Also Gyorgy and Goldblatt[44] had found that ceroid was appreciably diminished in experimental dietary cirrhosis if no cod liver oil was used in the diet

Some renewed interest in ceroid was aroused by Hartroft [0] when in a discussion of the histological picture of the livers in choline deficient rats he suggested that ceroid pigment appears to be formed by the interaction of red cells and fat Again in 1951[61] he described how the red blood cells which enter ruptured fatty cysts undergo a series of morphologic changes and may become closely bound to ceroid so that they are often indistinguishable from this pigment

The only fair assumption at the moment would seem to be that the importance and significance of ceroid is still in considerable doubt

NECROSES OF THE LIVER

On a purely anatomical basis necrosis of the liver can be divided into two main groups The first of these is *zonal necrosis* the second *massive necrosis* The zonal necroses can be further subdivided into *central necroses* *periportal necroses* and *midzonal necroses* In the zonal form each lobule is affected in a similar manner and to a similar extent Massive necrosis on the other hand is necrosis affecting the liver cells in one or more complete lobules and frequently involves large areas of the liver but may have other large areas intact As emphasized by Himsworth[61] this separation of necroses into zonal and massive forms is important as it allows us to think more clearly in interpreting the end or healed stages of these lesions

From the etiological standpoint the zonal necroses are to a large extent caused by liver poisons and can be produced experimentally by such substances

Neal[10] in 1944 summarizes the various substances which are used industrially which are considered to be hepato toxic

(1) The chlorinated aliphatic and aromatic hydrocarbons as represented by chloroform carbon tetrachloride trichlorethylene dichlorbenzene diphenyl chloride chlorinated naphthalenes and chlorinated phenols

(2) Aromatic amines such as toluylene diamine and others
(3) Aromatic nitro compounds such as di and tri nitrotoluene
(4) Aliphatic thiocyanates, especially of higher aliphatic hydrocarbons
(5) Possibly, also unsaturated aliphatic hydrocarbons such as propene

Carbon tetrachloride and trichlorethylene are important as solvents Diphenyl chloride and chloronaphthalene are used in insulating materials Fungicides and insecticides contain tetrachlorophenol, thiocyanates and DDT The aromatic amines are important as intermediates in the dye industry, and the aromatic nitro compounds play an important role as explosives

Certain naturally occurring poisons have similar effects Among these should be mentioned the endotoxin of Proteus Vulgaris extracts of certain algae, and certain poisonous mushrooms such as the Amanita phalloides [61]

Some of these substances in single doses produce a central necrosis Such are carbon tetrachloride, chloroform tannic acid and mushroom poisoning In contrast allyl formate, phosphorus and the endotoxin of Proteus Vulgaris give rise to periportal necrosis [61]

Our knowledge as to the etiology of massive necrosis has increased greatly in recent years In the past the accepted concept of the cause of acute yellow atrophy has been that it was toxic in origin Large doses of the substances just considered above can produce massive necrosis, but now, due largely to the pioneer studies of Glynn and Himsworth[41] on the one hand and Lucke and Mallory[84] (1946) on the other we know that this picture, usually known as acute yellow atrophy in this country can be produced by either nutritional deficiency or viral hepatitis

This latter aspect has been discussed under viral hepatitis, and the nutritional part of the picture will now be taken up in some detail Such a large volume of work has been carried out on the subject of the effect of nutrition on the liver that it is impracticable to try to trace the development of our knowledge step by step, but a review of this will be given as presented by Himsworth[61] in his Lowell Lectures in 1947 To Himsworth should go a large part of the credit for increasing our knowledge in this field not only because of the outstanding, original work carried out by him and his collaborators but also because he has reviewed the literature on the subject very thoroughly and in this way has markedly clarified the whole problem

For many years it has been suspected that diet in one way or another influenced the effect of toxic substances on the liver It was not until 1935 due to the study of Weichselbaum [148] that any evidence was presented which proved that liver injury itself could be produced by dietary deficiency He demonstrated that on diets low in casein rats suddenly became ill and died with what he called hemorrhages in the liver In 1939 Gyorgy and Goldblatt[4] studied hepatic injury of the rat liver arising on a nutritional basis They produced fatty degeneration focal and massive necrosis hyperemia and hemorrhage and in some rats a perilobular and condensation fibrosis on deficient diets They felt that the changes produced could be correlated to a deficiency of a part of the vitamin B complex It seems probable that the hemorrhages' of Weichselbaum were areas of massive necrosis

In 1944 Glynn and Himsworth[41] brought forward evidence that there were two types of liver injury that could be produced experimentally by dietetic injury The first of these was acute massive necrosis which is similar in every way with

the acute yellow atrophy seen in man The rats surviving this massive necrosis developed what is termed by Himsworth postnecrotic scarring and nodular hyperplasia This is the type of cirrhosis called by F B Mallory[9] "healed acute yellow atrophy" or "toxic cirrhosis" The second type of injury was a massive fatty infiltration leading in time to the diffuse hepatic fibrosis of Himsworth a picture which is usually known as Laennec's " ' alcoholic," or "portal cirrhosis This second type of injury will be discussed more fully later under fatty infiltration and cirrhosis

The earlier literature on the pathogenesis of dietetic massive necrosis of the liver is voluminous and confusing It is summarized by Himsworth[61] by stating essentially that a comparison of the diets used in its production show that it is not due to a deficiency of fat, carbohydrate minerals choline thiamine pyridoxine riboflavin, pantothenic acid or vitamins A and D It is not due to an excess of fat as claimed by some but does correlate with the intake of protein not the proportion in the diet but the absolute amount eaten each day After considerable research on the amino acids in the protein molecule, the important deficiency was narrowed down to methionine and cystine Further narrowing down of the field was difficult because of two features The first of these was that methionine is converted in the body to cystine The second arose from the fact that it was discovered that an inadequate supply of tocopherol an alcohol which has the properties of vitamin E and can be isolated from the oil of the germ of wheat might play an important role in the production of massive necrosis Himsworth[61] concludes (p 60) that it thus appears that dietetic massive necrosis is essentially due to deficiency of cystine but that another factor an inadequate supply of tocopherol, may sensitize the animal to the effects of such a deficiency "

Whether or not massive necrosis and its sequelae occur frequently in man as the result solely of malnutrition is still somewhat debatable There seems little doubt that the lesion is unusually common in tropical and subtropical races which are subject to malnutrition There is also a definite possibility that there may be a nutritional factor in the massive necrosis occurring in pernicious vomiting of pregnancy and that seen in viral hepatitis In toxic necrosis also the nutritional factor may be an important one Miller and Whipple[103] (1940) have demonstrated that chloroform is much more toxic to the liver in animals with protein depletion Furthermore, sulphur containing amino acids—methionine cystine and cysteine, but not choline -afforded definite protection against chloroform to these protein depleted dogs Gyorgy *et al*[46] (1946) had similar results in rats exposed to carbon tetrachloride vapor Animals on a low protein diet were more susceptible Methionine could be used as a substitute for protein and was equally effective in preventing liver damage

Pathological Features of Liver Necroses -The *zonal necroses* are to a large extent a microscopic rather than a gross finding Central necroses may be visible as yellowish depressed areas in the centers of the lobules With central hemorrhagic necrosis the central areas are again depressed and red purple in color The histological picture of zonal necrosis will depend on the age of the process and the type of zonal necrosis present In central necrosis fatty degeneration is apt to precede the necrosis and show itself as numerous fine fat droplets in the centers of the lobules This is followed by necrosis of the central cells polymorphonuclear leukocyte infiltration and later by histiocytic infiltration with removal of the necrotic cells

If the necrosis is hemorrhagic in type as is seen in chronic passive congestion of the liver, extravasation of red blood cells will take place into and around the necrotic liver cells In periportal necrosis the microscopic picture is similar to that described for central necrosis save for the fact that the necrotic tissue lies at the periphery of the lobule Midzonal necrosis is a curious phenomenon where the necrotic liver tissue lies approximately midway between the central veins and portal areas

The most important feature to be emphasized about all these forms of zonal necrosis is that, inasmuch as only part of the lobule is involved and the supporting framework, composed of sinusoids reticulum and connective tissue does not collapse rapid regeneration with a complete restitution to normal within two or three weeks usually occurs

Massive necrosis has a very characteristic gross picture in both the experimental and human lesions although this again varies with the age of the process As described by Himsworth,[61] if the animals with experimental dietary necrosis are killed within an hour of when they first become sick the livers are swollen, generally vermillion in color but spotted on their surface with dark red flecks As is readily understood this stage is rarely, if ever seen in the human, as it would necessitate opening the abdomen or at least doing a peritoneoscopic examination in a sick but living patient Twelve to twenty four hours later the rat livers have a very similar picture to that known to most pathologists as acute yellow atrophy in man Two to three days to a week later the yellow color has become more dull and large depressed dark red areas have appeared This corresponds to the subacute red atrophy seen in humans Weeks later the yellow areas become the color of normal liver substance and the red areas turn into depressed irregular scars—the healed acute yellow atrophy or toxic cirrhosis of man

Microscopically the experimental lesions are very similar to the stages seen in the human acute yellow atrophy In the earliest stage described grossly the portal vessels are congested the portal areas are surrounded by pools of blood The liver cells appear severely damaged or actually necrotic At twenty four to forty eight hours in large areas of the liver an obvious necrosis of all the liver cells but not of the Kupffer cells or the contents of the portal areas has developed By the time that the subacute red atrophy stage has been reached histiocytes have removed the necrotic liver cells and the sinusoids are congested with red blood cells

Here in contrast to what was described in zonal necroses the supporting reticulum framework of the lobule collapses bringing adjacent portal tracts closer together There is also marked proliferation of bile ducts but inasmuch as these are incapable of forming liver cells and there are no liver cells left in the lobule a restitution to normal of the involved areas is impossible

CIRRHOSIS

(a) Classification To anybody interested in diseases of the liver cirrhosis has always been one of the most fascinating and important lesions One of the biggest problems in relation to cirrhosis has always been that there has been no uniformity of opinion as to exactly what constitutes cirrhosis of the liver or as to the etiology and classification of the lesions usually called cirrhosis

Even the term cirrhosis is considered by many a misnomer The term was originated by René Theophile Hyacinthe Laennec to describe the yellow but also fibrosed and nodular livers that he saw The derivation is from the Greek work "kirrhus" meaning yellow or orange colored, and implies nothing as to fibrosis or nodular regeneration Himsworth [2] states definitely (p 15), "Fibrotic conditions in the liver are due to different entities and it would be better to drop the name cirrhosis and classify the fibrotic state as definite and different entities"

Nevertheless over the period of many years cirrhosis has become recognized as a very definite type of process involving the liver having a definite clinical symptomatology, and producing a well known syndrome The term has been too thoroughly incorporated into the medical minds and the medical literature to make it worth while to try to change the name merely on the basis of a poor derivation By custom and usage it now means a lesion of the liver resulting from an acute or chronic progressive necrosis of liver cells which is associated with a real or apparent increase in the connective tissue Although in most cirrhotic livers there is also a nodular regeneration of liver cells this is not always true and therefore, it should probably not be included in a definition of the process

The classification of cirrhosis is also a difficult problem Various methods have been tried such as naming the type of cirrhosis after the individual who first described it Thus we have the terms Laennec's cirrhosis and Hanot's cirrhosis Himsworth states with considerable justice that Laennec's name should not be immortalized as a result of his studies on cirrhosis as he wrote but briefly on the subject Hanot's description of the type of cirrhosis named after him is so inadequate that nobody really knows the exact type of lesion that he was describing

Another method of classification has been to base it purely on morphology The older literature contains numerous references to atrophic cirrhosis and hypertrophic cirrhosis Frequently the terms monolobular cirrhosis and multilobular cirrhosis were also used More recently the term portal cirrhosis has been coined on the, now provenly mistaken basis that the fibrosis originated in the areas around the portal tracts Even the classification offered by Himsworth is essentially a morphological one He avoids the term cirrhosis but feels that what he considers as fibrotic processes of the liver can be divided into two main groups The first of these he calls diffuse hepatic fibrosis and puts into this group all cirrhoses in which the fibrosis is diffusely and more or less evenly found throughout the liver The second he calls postnecrotic scarring and in this group includes all cirrhoses in which there are large areas of scarring as well as large areas in which normal liver tissue still remains

It seems that the time has arrived when a more satisfactory solution to this problem of classification should be attempted Such a classification should use a nomenclature that combines morphology as nearly as possible with some concept of etiology A classification along these lines was always taught by F B Mallory and is discussed by him in the Shattuck Lectures[23] delivered in 1932 The five main types of cirrhosis in the F B Mallory classification were

(1) Healed acute yellow atrophy or toxic cirrhosis
(2) Alcoholic cirrhosis
(3) Pigment cirrhosis
(4) Biliary cirrhosis
(5) Syphilitic cirrhosis

If this classification is modified to the extent of incorporating into it the new ideas of etiology as expressed by Himsworth, the result should be much more practical and expressive. The brilliant studies of Best, Himsworth and others have undoubtedly clarified our concepts of the etiology of acute yellow atrophy and the resultant healed acute yellow atrophy. They have also made obsolete the term alcoholic cirrhosis.

To alter this along the lines suggested above it would be necessary only to change the healed acute yellow atrophy to "Focal Postnecrotic Cirrhosis" and the alcoholic cirrhosis to "Diffuse Fatty (Nutritional) Cirrhosis." With the first, if the probable etiology can be determined from the history, this could be indicated by putting it in parentheses after the diagnosis. Thus if it were considered nutritional in origin, the diagnosis would read "Focal Postnecrotic Cirrhosis (Nutritional)."

Some of our present concepts about these types of cirrhosis will now be discussed.

(b) **Focal Postnecrotic Cirrhosis.**—Under this title the form of cirrhosis called postnecrotic scarring by Himsworth[61] and toxic cirrhosis or healed acute yellow atrophy by Mallory will be discussed. It is the type of cirrhosis which is found after the massive hepatic necrosis of Himsworth or the acute yellow atrophy of most other authorities. As has been pointed out in the discussion of the hepatic necroses, such massive necrosis can be caused by a variety of factors which injure the liver. The morphological picture found in the healed stage is the same regardless of the cause of the original injury. The diagnosis of the etiology must depend on a careful analysis of the history of the patient.

The experimental production of massive necrosis by Himsworth and others has already been described in some detail under necroses. Here it should suffice to repeat Himsworth's[61] conclusions that dietary massive necrosis of the liver is essentially the result of a deficiency of cystine in the diet. Those animals that survived the massive necrosis produced by this method developed the form of liver cirrhosis under discussion.

How frequently focal postnecrotic cirrhosis in man is the sole result of such cystine deficiency is still open to argument, but there seems to be no doubt that such a deficiency plays a very important role in the development of this lesion, at least as a contributory factor. As has been described under viral hepatitis, fatal cases develop a massive necrosis of the liver. It is obvious that any individual who survives such a massive necrosis should develop a focal postnecrotic type of cirrhosis. To date we have not seen or heard of a case where hepatitis was satisfactorily proven as a cause of such cirrhosis. The answer to this problem must await more and better authenticated cases.

As massive necrosis is known to occur as a complication of pernicious vomiting of pregnancy, *as a result of mushroom poisoning*, and the use of drugs such as chloroform and arsphenamine, it is also probable that focal postnecrotic cirrhosis might occur in such patients if they survived the acute necrotic process. In practically any of these conditions, as pointed out by Himsworth, cystine deficiency might be an etiological factor due to the fact that because of their illness the patients were unable to eat or at least absorb a sufficient amount of the proper nutriment from their diets. As he also indicates, adequate diets can become inadequate when *for some reason* such as hyperthyroidism or pregnancy the demands for nutrition of the liver cells become greater than the nutrition supplied

Morphologically the gross and microscopic pathology of the liver in focal post necrotic cirrhosis is similar in the experimental and the human lesions Grossly these livers tend to be small Of 44 cases in man so diagnosed at the Boston City Hospital, 21 or almost 50 per cent weighed less than 1,000 grams They show two other very characteristic features The most striking of these is the presence of large atrophic scars These areas are depressed below the surface and have a slightly roughened surface In color they are the same as the surrounding liver tissue Very frequently the left lobe is more involved than the right, which has been explained by Himsworth to come about as the result of the distribution of the portal blood stream (Fig 30) The second feature is large nodules of re generation These may vary from 1 to 6 or more centimeters in diameter

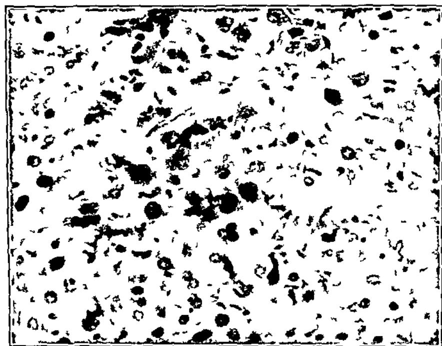


FIG 29 —*Benign Viral Hepatitis Hematoxylin Eosin* × 400 Bile stasis evidenced by bile canaliculi filled with inspissated bile

Several histological features are necessary in order to make a histological diag nosis The most important of these, according to F B Mallory, is to find large areas involving multiple liver lobules in which no liver cells are present and only bile ducts sinusoids and thickened connective tissue remain (Fig 31) An other feature emphasized by Himsworth[61] is to find in areas away from the scars completely normal liver lobules Microscopic examination of the nodules of regeneration shows the lobules to vary in size and in the location of the hepatic venules which are apt to be somewhat eccentric in position

In the scarred areas there is evidence of a proliferation of bile ducts Although connective tissue is always much more obvious in the scars than in the normal liver tissue most authorities agree that this is not the result of a proliferation of new connective tissue but represents a condensation of that connective tissue

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present before the necrosis occurred It is much more obvious because the connective tissue cells have laid down large amounts of collagen

Clinically this type of cirrhosis results in pretty much the same type of clinical symptomatology as does any other type of cirrhosis There is portal hypertension splenomegaly ascites and esophageal varices which tend to rupture It should be emphasized that it is the result of one episode of acute necrosis of liver cells rather than a chronic and progressive injury to them

(c) **Diffuse Fatty (Nutritional) Cirrhosis** —Other names for this type of cirrhosis are Laennec s cirrhosis portal cirrhosis, alcoholic cirrhosis (Mallory) and diffuse hepatic fibrosis (Himsworth) This type of cirrhosis is now known to follow and be the result of prolonged and excessive fatty infiltration Therefore the etiology of the cirrhosis is the same as that of fatty infiltration This has already been discussed under fatty infiltration For years investigators of the problem were misled because it is this type of cirrhosis that is found in chronic alcoholics Therefore the early investigators thought that alcohol *per se* or at least some contaminant of alcohol must be the cause of the liver injury and the consequent cirrhosis It was only after it was pointed out by Chaikoff and Connor[16] that long continued fatty infiltration produced cirrhosis and it was also demonstrated by Best[7] and others that dietary insufficiency could produce fatty infiltration that it was realized that the cirrhosis seen in chronic alcoholism is the result, not of the alcohol itself, but of the dietary insufficiency from which a large proportion of chronic alcoholics suffer As Himsworth[61] so aptly says there are no lipotropic factors in alcohol when taken in excess it produces a gastritis which impairs the appetite and it is expensive as are foods rich in lipotropic substances

Experimentally diffuse fatty nutritional cirrhosis has been produced in a variety of ways Thus as described under fatty infiltration it can be produced by the removal of the pancreas or by diets deficient in choline or methionine It has been produced in dogs which were fed a high fat diet and also subjected to repeated bleedings (Holman [64] 1945)

There is abundant evidence that this cirrhosis occurs in man as a result of a similar nutritional deficiency Its relation to chronic alcoholism has been explained above It is however common in the tropics where alcoholism is rare and deficient diets common Its occurrence in such regions can be correlated with poverty and in turn with malnutrition Although the scale of living in Western Civilization is on the whole too high for such an etiology to be common it did as described by Himsworth [61] occur in recent times of famine in Europe During World War II the hospitals in Budapest were filled with children with large fatty livers These livers returned to normal size when milk and meat became available Three of the children died of other causes later and were found at autopsy to show diffuse fatty (nutritional) cirrhosis

The gross and microscopic picture of these livers will vary with the stage that the process has reached when the liver is seen Grossly in the earliest stage nothing but the signs of massive fatty infiltration will be seen The livers will be large sometimes weighing as much as 5 000 grams swollen and yellow The surface will be smooth and no evidence of fibrosis will be seen on gross section In contrast to this in the late stage the liver will be contracted and diffusely and finely granular The granules will vary in size but rarely reach more than 1 centimeter in diameter Even grossly fibrosis can be made out at the periphery

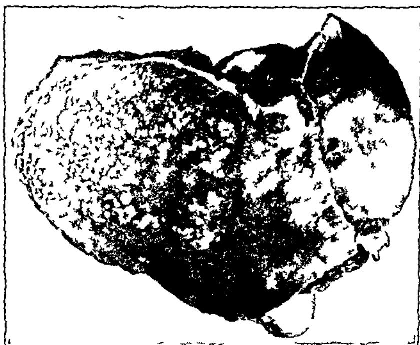


Fig. 30.—*Focal Postnecrotic Cirrhosis.* An atrophic cirrhotic liver. The left lobe is completely atrophic save for four nodules of regeneration. The right lobe is also atrophic but contains multiple nodules of regeneration.

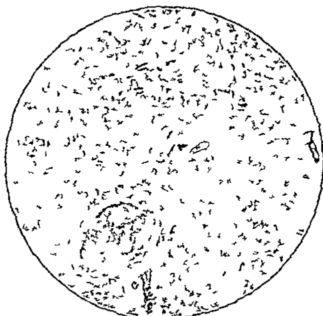


Fig. 31.—*Focal Postnecrotic Cirrhosis. Phloxine Methylene Blue* × 10. Area of scarring to show condensation and thickening of the connective tissue and bile duct proliferation. Although several lobules are present only liver cells in field are in a nodule of regeneration.

students of the subject agree that the fibrosis is not restricted to the periphery of the lobule and in fact often begins around the hepatic or central veins

In diffuse nutritional cirrhosis in contrast to postnecrotic cirrhosis the fibrosis is the result of a chronic progressive necrosis of liver cells rather than of one extensive acute episode of necrosis All areas of the liver show essentially a similar process in diffuse fatty (nutritional) cirrhosis, whereas in postnecrotic cirrhosis large areas show scarring and atrophy and areas of normal intact liver tissue can also be found

These differences between those two types of cirrhosis are not only important from the standpoint of diagnosis but also from the standpoint of possible therapeusis It is obvious that once the massive necrosis which results in postnecrotic scarring has taken place nothing can be done, other than to attempt to handle the complications that arise from this necrosis and resultant fibrosis as well as possible The situation with fatty nutritional cirrhosis is quite different In the early stages where only fatty infiltration is present, proper diets containing adequate protein and lipotropic substances can result in a restitution to normal This has been demonstrated by Hartroft[49-51] and others in the experimental lesions It is probable that once extensive fibrosis has developed, the process becomes more or less irreversible but even here a reduction of the fat content of the remaining liver cells should be beneficial

(d) **Pigment Cirrhosis** —Pigment cirrhosis has long been known as a type of cirrhosis occurring in association with hemochromatosis Morphologically the picture is well known Grossly the liver will vary in appearance with the stage that the process has reached In the early stage the liver is large smooth, and a deep chocolate brown in appearance Even on cross section no fibrosis is distinguishable grossly In contrast to this in the late stages the liver is somewhat contracted and diffusely and finely nodular The size of the nodules varies but similar to the picture in diffuse fatty nutritional cirrhosis none are more than 1 centimeter in diameter The color is a deep chocolate brown and frequently small pale nodules of regeneration can be seen All intervening stages between the early and the late may be found

Microscopically in the early stage the most striking feature is the large amount of hemosiderin pigment in the liver cells the Kupffer cells the bile duct epithelium and the connective tissue of the portal areas This pigment is golden brown in color with phloxine methylene blue and hematoxylin eosin stains but will stain a deep blue if an iron stain using ferrocyanide of potassium and hydrochloric acid is used In addition to this pigment smaller amounts of a second pigment hemofuscin are seen This again may be found in any of the cells in the liver but is usually most prominent at this stage in the bile duct epithelium and connective tissue of the portal tracts The hemofuscin does not give a positive iron reaction it is blue green in a phloxine methylene blue stain and stains a bright red in Mallory s[86] special stain for hemofuscin In this early stage a slight increase in connective tissue most prominent around the periphery of the lobules is found

In the late stage the microscopic picture is considerably altered The liver tissue shows a diffusely distributed fibrotic process by which it is divided up into many islands which vary in size There is evidence of a proliferation of bile ducts in this fibrous tissue Most of the liver cells contain large amounts of hemosiderin pigment Some islands of liver cells contain little pigment and on close examination show evidence of regeneration such as mitotic figures As these areas of

of the granules Usually the color will be somewhat yellow due to a small amount of remaining fat These are the two extremes, and every variety of intervening stages can be found

Microscopically the striking feature to be seen in the early stages is fatty infiltration Most liver cells will be distended with a large fat vacuole shoving the cytoplasm to the periphery Fatty cysts may be seen as described by Hartroft [51] In man the so called "alcoholic hyaline" of Mallory [95] is apt to be a prominent feature (Fig 32) This starts as hyaline droplets in the cytoplasm of the liver cells The droplets fuse to form an irregular skein or network around the nuclei This hyaline material takes an acidophilic stain and would seem to be a sign of degeneration, as the cells which contain it often become necrotic The signifi

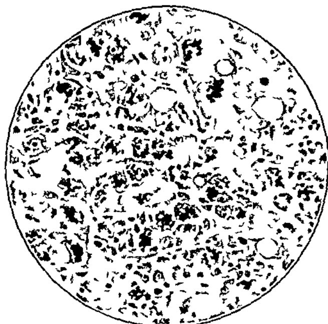


FIG 32 —*Fatty Nutritional (Alcoholic) Cirrhosis Phloxine Methylene Blue* × 300 Early stage showing fat and large amounts of alcoholic hyalin

cance of "alcoholic hyalin" is somewhat debatable If suitable stains are available and the observer knows what to look for it can be found in almost any cirrhotic liver of this type F B Mallory felt that it had some relation to the alcohol consumed It is however reported in experimentally produced lesions on deficiency diets and in the livers of children with this type of cirrhosis who consumed no alcohol

The origin of the fibrosis that is found in these livers is also somewhat controversial F B Mallory always maintained that it was the result of condensation of the stroma that supported the liver cells which had become necrotic and were removed He also claimed that the only new connective tissue formed was that in the stroma of the regenerating cells When these in turn became necrotic this newly formed stroma became condensed and prominent Hartroft [51] (1951) suggests the same origin of the fibrous tissue in the experimental cirrhosis All

the parenchymatous tissue The underlying anemia and the somewhat frequent transfusion reactions are thought to act as predisposing factors for the development of this exogenous hemochromatosis

The possible relation between this type of hemochromatosis and the more usual type is interesting to consider It of course immediately suggests that the usual type might be the result of excessive and abnormal destruction of red blood cells This has however been pretty completely ruled out because there is no hematological evidence of a hemolytic anemia red cell hyperplasia of the bone marrow or increased production of bilirubin in the usual hemochromatotic patient

Extensive studies on iron metabolism have been carried out by Finch *et al*[33,34,35] in 1949 and 1950 In 1949 Kinney Hegsted and Finch[7] reported that as a result of feeding rats a corn grit diet containing large amounts of ferric citrate excessive amounts of iron were deposited in their livers It was also possible to obtain excessive iron deposits in the livers of animals receiving a normal diet by adding large amounts of iron salts to the diet No appreciable cirrhosis seems to have been produced Large amounts of hemosiderin were deposited

(e) **Biliary Cirrhosis** —In 1931 F B Mallory[94] described two types of cirrhosis which were biliary in type The first of these was called colon bacillus cirrhosis on the basis that the infection was usually caused by that organism As time went on other organisms were found to cause the lesion and the term infectious cirrhosis was substituted for the colon bacillus cirrhosis Infectious cirrhosis is due to the invasion of the bile ducts (cholangitis) rarely the portal veins (pylephlebitis) by organisms The infection tends to spread to the liver tissue adjacent to the portal areas Here it results in necrosis of liver cells and eventually in fibrosis and proliferation of bile ducts

The second type was called by Mallory obstructive cirrhosis In this type as a result of prolonged obstruction of the larger bile ducts the smaller bile ducts in the portal areas proliferate and the connective tissue that subtends them also proliferates This leads to a diffuse type of cirrhosis of the liver which arises in the periphery of the lobules MacMahon and Mallory[88] (1929) reported 30 cases of such cirrhosis in which there was no superimposed infection Ten of these cases were infants and 20 adults The most common cause of the lesion in infants is a congenital malformation of the bile ducts whereas in the adult it results from tumors concretions and inflammatory stricture At the same time MacMahon Lawrence and Maddock[87] reported the experimental production of obstructive cirrhosis by tying the common bile duct in guinea pigs By the end of two weeks considerable cirrhosis similar to that described above was found

From the descriptions of these two types of cirrhosis it can be seen that a cirrhosis starting at the peripheries of the lobules and eventually tending to surround each liver lobule by a zone of connective tissue and proliferating bile ducts can occur (Fig 33) As with the kidney in obstruction of the ureter in the liver any prolonged obstruction of the bile ducts is apt to be complicated by a superimposed infection of the biliary system Therefore in practice combined lesions of infectious and obstructive cirrhosis are usually found and for this type of combined lesion the term biliary cirrhosis is used

In recent years Spink[136] in describing the focal granulomatous lesions seen in the liver in Brucellosis suggested that in healing a cirrhosis of the liver might occur Also it has been reported by Symmers[138] in 1904 that the ova of *Schistosoma mansoni* are the chief cause of cirrhosis of the liver in Egypt, and similar

regeneration become older according to the opinion of F B Mallory, hemofuscin is present first in those regenerating liver cells and this is later transformed into hemosiderin As described in the early stage, both hemosiderin and hemofuscin are found in the connective tissue and bile duct epithelium throughout the liver

The genesis of the connective tissue is probably similar to that already described in diffuse nutritional fatty cirrhosis Liver cells heavily laden with pigment become necrotic and are removed This suggests that the pigment produces the necrosis, but this is unproven Nodules of regenerating liver cells compress and condense the supporting stroma that is left behind New stroma is formed in the regenerating nodules, and when these newly formed liver cells in turn become necrotic more connective tissue stroma is condensed

The etiology of pigment cirrhosis is still to a large extent unsolved In a series of three papers[91–93] (1921, 1925 and 1926) F B Mallory considered the cause to be *chronic poisoning with copper He based this belief on the production of hemo* chromatosis in rabbits and monkeys by chronic poisoning with copper salts and by staining granules in the liver cells of human cases with a stain (unripened hemotoxylin) which he considered specific for copper Most pathologists today would agree that the rabbit lesions were very similar, if not identical, to those found in human hemochromatosis Schonheimer and Oshima[1 9] (1929) reported the copper content of normal livers as 1 3 to 3 9 mg /1000 grams of liver and from 9 86 to 63 2 mg in hemochromatotic livers Herkel [8] (1930) found the normal range to be 6 5 to 36 1 mg with 42 to 384 mg in hemochromatosis Herkel also found in unpigmented types of cirrhosis a range from 67 6 to 379 mg Other investigators, too, have been unable to reproduce Mallory's results and the theory that chronic copper poisoning is the cause of hemochromatosis is not widely accepted

Many other theories as to the etiology of hemochromatosis have been offered Rosenthal[127] (1932) feels that it results from an inability of the liver cells to reduce ferric to ferrous iron Sheldon[13] (1935) considers the disease caused by an inborn error of metabolism Gillman *et al* [39] (1945) claim hemochromatosis to be a manifestation of chronic malnutrition, and that a frank pigmentary cirrhosis is found in 12 5 per cent of pellagrins They feel that both the hemosiderin and hemofuscin have a common origin from mitochondria and propose the names ' cytosiderin for the former and cytolipochrome for the latter This study was carried out in Johannesburg South Africa on the native Bantu workers It is very difficult to evaluate it as this type of lesion seems restricted to South Africa and we have never seen similar material

In recent years an interesting observation has been made on individuals who have been given a large number of transfusions usually 100 or more They develop a process which is probably identical with hemochromatosis Schwartz and Blumenthal[130] (1948) present 5 cases and review the literature on this syn drome They suggest the name exogenous hemochromatosis for this type of lesion Their 5 cases were all females who received transfusions for severe anemia Eight similar cases were added from the literature *In 12 of the 13 of these cases* on which an autopsy or a liver biopsy was performed hemosiderosis and fibrosis of the liver was found The presence or absence of hemofuscin is not mentioned but it has been seen by us in at least one similar case Schwartz and Blumenthal postulate that the hemochromatosis developing in these patients is the end result of the deposition and subsequently irritating action of excess amounts of iron in

the parenchymatous tissue. The underlying anemia and the somewhat frequent transfusion reactions are thought to act as predisposing factors for the development of this exogenous hemochromatosis.

The possible relation between this type of hemochromatosis and the more usual type is interesting to consider. It of course immediately suggests that the usual type might be the result of excessive and abnormal destruction of red blood cells. This has, however, been pretty completely ruled out because there is no hematological evidence of a hemolytic anemia, red cell hyperplasia of the bone marrow or increased production of bilirubin in the usual hemochromatotic patient.

Extensive studies on iron metabolism have been carried out by Finch *et al* [33,34,35] in 1949 and 1950. In 1949 Kinney, Hegsted and Finch[75] reported that as a result of feeding rats a corn grit diet containing large amounts of ferric citrate, excessive amounts of iron were deposited in their livers. It was also possible to obtain excessive iron deposits in the livers of animals receiving a normal diet by adding large amounts of iron salts to the diet. No appreciable cirrhosis seems to have been produced. Large amounts of hemosiderin were deposited.

(e) **Biliary Cirrhosis.**—In 1931 F. B. Mallory[94] described two types of cirrhosis which were biliary in type. The first of these was called colon bacillus cirrhosis on the basis that the infection was usually caused by that organism. As time went on other organisms were found to cause the lesion and the term infectious cirrhosis was substituted for the colon bacillus cirrhosis. Infectious cirrhosis is due to the invasion of the bile ducts (cholangitis), rarely the portal veins (pylephlebitis), by organisms. The infection tends to spread to the liver tissue adjacent to the portal areas. Here it results in necrosis of liver cells and eventually in fibrosis and proliferation of bile ducts.

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lesions are described with *S. haematobium* or *S. japonicum* in southern Africa, China, Japan and elsewhere. Reports from Prussia, Siberia, French Indo China and to a lesser extent India describe a similar cirrhosis caused by the liver flukes *Opisthorcis felineus* and *Clonorchis sinensis* (Berman[9] 1951). All of these would seem to be types of biliary cirrhosis.

In 1948 MacMahon[83] described a type of cirrhosis previously called "Xanthomatous Biliary Cirrhosis." The study was based on liver biopsies from 4 patients selected as showing signs and symptoms of the syndrome xanthomatous biliary cirrhosis by Dr. S. J. Thannhauser. Ten years previously Thannhauser and Magendantz[139] had proposed the name of "Xanthomatous Biliary Cirrhosis" for the liver changes found in patients who had had prolonged jaundice, an enlarged palpable liver, cholesterol blood levels above normal and xanthomatous change on the hands and bodies. These authors had considered the specific histologic

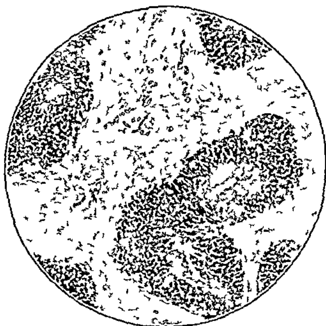


Fig. 33.—*Biliary Cirrhosis. Phloxine Methylene Blue* × 20. Liver lobules surrounded by a zone of dense fibrous tissue showing bile duct proliferation and inflammatory infiltration.

lesion of the liver to be xanthoma cells and increased fibrous tissue in the walls of the intrahepatic system of bile ducts. They considered these liver lesions to be a manifestation of a hereditary and systemic disorder which they called essential *hypercholesteremic xanthomatosis*.

MacMahon feels that there is no basis for considering this syndrome as a manifestation of a single and specific disease of the liver or for including it in the family of hereditary essential hypercholesteremic xanthomatoses. The name biliary xanthomatosis has been suggested because an early jaundice and the presence of xanthomatoses are the striking clinical features. In a later publication MacMahon and Thannhauser[90] suggest that the hepatic disease may precede the xanthomatosis.

(f) **Syphilitic Cirrhosis** —According to the concept of F B Mallory this could be divided into two types (1) congenital syphilitic cirrhosis and (2) the Hepar Lobatum of tertiary syphilis The gross picture of congenital syphilitic cirrhosis is a somewhat enlarged smooth, firm liver Microscopically there is found a proliferation of fibroblasts beneath the Kupffer cells diffusely throughout each and every lobule With Levaditi s stain Treponema pallidum in large numbers can be found in this connective tissue, and the fibrosis probably represents a reaction to them

The pathology of Hepar Lobatum is well known Grossly the liver is divided up into many coarse lobules by deep scars The scars result from the formation and healing of gummata If the process is an active one, gummata may be found in the scar tissue Histologically sections through the scars show dense scar tissue with complete disappearance of normal liver tissue Evidence of a healing gumma may be seen In sections from the centers of lobules normal liver tissue with normal lobulation is found

REGENERATION

The capacity of the parenchymal liver cells to regenerate is astonishing This has been proven experimentally by extirpating large portions of the liver and observing how rapidly it returned to a normal size Von Meister[144] (1894) reported the removal of 75 per cent of the liver in rats rabbits and dogs and this was followed by restoration of the liver to as much as 80 per cent of its original weight Mann and Magath[99] (1922) had similar results in dogs The regeneration took forty five to sixty days It occurred more rapidly in young strong animals Fishback[35] (1929) working with dogs found that this regeneration would take place repeatedly when the same operation was done on the same animal In 1931 Higgins and Anderson[60] removed 70 per cent of the liver in white rats and reported that the remaining fragment doubled in weight in seventy two hours In a similar experiment also using rats Brues *et al* [1] in 1936 found that after removal of 70 per cent of the liver what remained of the liver increased 50 per cent in forty eight hours Gross and histological examinations of these regenerated masses demonstrated them to be the result of budding from the remaining fragment Therefore, histological sections showed them to be composed of normal appearing lobules with central veins and portal areas at the periphery

Probably more important than this ability of the liver of a normal animal to regenerate is a consideration of the factors which affect and limit regeneration If in the dog (Mann and Magath[99] 1922) an Eck fistula is made diverting the portal circulation from the liver, and then a portion of the liver is removed there will be very slight if any regeneration of the remaining fragment The conclusion was drawn from this experiment that the ability of liver tissue to regenerate was dependent on an intact portal circulation and was somewhat proportional to the nutrition brought by that circulation This concept is further strengthened by an experiment in which it was shown that ligation of one of the two main branches of the portal vein led to atrophy of the lobe supplied by the ligated vein and a hypertrophy of the other lobes (Rous and Larimore[124] 1920)

Pathologically it is well known that there is little evidence of liver cell regeneration in biliary cirrhosis associated with biliary obstruction It was shown by Cameron[13] (1935) that, if he transplanted liver tissue in which the bile ducts had

been tied it did not grow as did liver tissue in which there was no biliary obstruction. Bile stasis, therefore seems to be unfavorable for the regenerative process.

In most forms of cirrhosis of the liver one of the striking features is regeneration. It is also known to occur rapidly following zonal necrosis. Consideration of the regenerative processes in these two conditions points out some of the factors regulating regeneration. *In the zonal necroses regeneration* replaces the cells lost as the result of the necrosis and a restitution to normal rapidly occurs. As there is always at least a rim of surviving liver cells on the periphery of the necrosis for new cells to arise from and a framework of reticulum and sinusoids for them to grow over this process is understandable.

After massive necrosis however there are large areas involving many liver lobules in which there are no surviving liver cells. Also the supporting framework of reticulum and sinusoids has collapsed and is distorted. Therefore regeneration must start at the periphery of the area of necrosis and without a normal supporting framework is unable to re form lobules similar to those present before the necrosis occurred. The result is large nodules of regenerated liver tissue which can be seen grossly and microscopically. This regenerated tissue does not have the uniform lobular pattern that is seen in normal liver tissue. The lobules vary in size and the hepatic venules are eccentrically placed. This distorted picture is probably explainable on the basis of pressure from the surrounding liver tissue particularly inasmuch as this contains connective tissue with increased amounts of collagen.

PRIMARY CARCINOMA OF THE LIVER

The chief developments in relation to tumors of the liver in recent years have been in the field of primary carcinoma of the liver. Here as in so many fields of pathology we are plagued by a multiplicity of names for the same types of tumor. Everybody agrees that there are two types of cells in the liver which may give rise to primary cancer of the liver. The first of these is the liver cell itself and the other is the cell that lines the bile ducts *i e* the bile duct epithelium. For the tumors arising from the liver cells the terms 'primary liver cell carcinoma' 'hepatoma' and 'hepatocellular carcinoma' are used. For those arising from the bile duct epithelium there is even a greater variety 'bile duct carcinoma' 'alveolar carcinoma' 'adenocarcinoma' 'cholangioma' and 'cholangiocellular carcinoma'. Such a large number of names is very confusing and the adoption of the terms hepatocellular carcinoma for the first and cholangiocellular carcinoma for the second as suggested by *Berman*[6] *(1951)* would simplify the situation.

One of the most interesting developments in this field has been a study of the geographical frequency and distribution of primary carcinoma of the liver. This has been recently done in a book by Charles Berman[6] (1951). He points out that there is a marked contrast in the incidence of these tumors in the different races of the world. *Whereas it is relatively uncommon among Western peoples* (European and American) it is seen relatively often and with roughly equal frequency in the African and in some Oriental races. Among Bantu (African) and Javanese males it is undoubtedly the most common visceral cancer encountered. He found that among 772 cancer cases in male Bantu laborers in the Witwatersrand gold mines in South Africa 670 cases or 86 8 per cent were primary cancers of the liver

102 cases or 13 2 per cent were carcinomas of other organs By far the most frequent histological type was the hepatocellular carcinoma In Javanese males almost 80 per cent of all carcinomas are primary in the liver

These facts about frequency and distribution immediately provoke interest in the possible relation of this to the etiology of these cancers This is also thoroughly discussed by Berman Ewing[3] (1940) states that cirrhosis is present in 85 per cent of liver cell cancers and 50 per cent of bile duct cancers Berman found in reviewing the literature that cirrhosis was present in 67 2 per cent of both forms of primary carcinoma Inasmuch as cirrhosis mostly on a dietary basis, is also very common in the areas where these tumors are frequent he feels that dietary causes are very important in relation to etiology Whether it is the diets *per se* or the regeneration provoked by the cirrhosis that is the more important is certainly open to argument Inasmuch as hepatocellular carcinoma occurs frequently in forms of cirrhosis such as pigment cirrhosis and postnecrotic cirrhosis where the dietary factors are either not the same as in fatty nutritional cirrhosis or not present at all the dietary factor by itself alone is perhaps not too important

Experimental primary liver cancer has been produced by a variety of substances The best known of these is ' butter yellow (p-dimethylaminoazobenzene) Kinosita[76 77] (1937 1940) succeeded in producing both types of tumor with this substance and studied the whole group of azo compounds The tumors have also resulted from dibenzanthracene (Ilfeld[4] 1936) selenium (Moxon and Rhian[104] 1943) carbon tetrachloride (Edwards and Dalton [9] 1942) and many other substances From this we may judge that primary carcinomas of the liver can be produced by carcinogenic substances but exposure to these substances by humans does not seem to have been very adequately proven

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(The photomicrographs of viral hepatitis are by the kindness of Dr L S Jolliffe)

Chapter

6

A SURVEY OF TECHNIQUES FOR THE HISTOCHEMICAL APPROACH TO PATHOLOGY

By J F A McManus M D

Introduction

This histochemical approach to pathology has two parts The first part is composed of the histochemical techniques which are simple exact, and sufficiently informative to be used in a laboratory of pathology In order to indicate these it is necessary to describe the materials studied the methods of their demonstration and results which can be expected These data are given in this essay or chapter

The second portion of the histochemical approach to pathology is one of concept or viewpoint It consists in thinking of the normal and diseased cells and tissue in terms of chemical constitution *in addition to* the morphological appearances The histology is necessary and primary since otherwise the histochemical pathologist becomes a micro chemist As such he is capable of perpetuating into pathology the purely biochemical viewpoints which many serious pathologists consider as a return to the humoral doctrines of Rokitansky It is hoped that the application of the techniques discussed in this chapter will allow the accumulation of data making up a histochemical pathology

In the discussion of the chemical features of tissues and the histochemical techniques formulae have been cut to the minimum The information which is furnished by the structural formulae does not appear necessary in much of this discussion Moreover some pathologists appear allergic to structural formulae Many develop an inferiority complex when confronted with them The chemists and biochemists who read this chapter will be I hope satisfied with the chemical terms and the chemical viewpoint

The introduction of histochemistry into biochemistry is in many ways a revolution which is of the same degree as the introduction of the microscope into anatomy That all biochemists will recognize this is too much to hope for just as it is beyond hope that all pathologists will recognize the value of histochemistry

It will be appreciated by many pathologists that the introduction of histochemistry into pathology is actually just an extension of the original morphological viewpoint with the addition of the chemical information which is furnished by the special techniques Fortunately enzyme methods are developing more rapidly than other methods in histochemistry This is desirable as concentrating on the dynamic approach to tissues in preference to the static classically morphological approach

It is probably along the lines of histochemistry that pathology is to develop Many pathologists would not agree with the few who believe that the progress

of pathology is in the direction of clinical patholgy with the pathologists to become a sort of assistant to the clinician Much progress can come and is coming out of histochemical pathology

Historical Notes —Histochemistry is a term which was introduced about the same time as the term histology [3] It seemed natural to the early workers to think of tissues from the aspect of chemical constitution quite as much as morphological appearance Techniques in the morphological field developed more rapidly than the chemical information As a result the chemistry of the tissues developed along the test tube line rather than the intra cellular values for which the early workers had hoped It is noteworthy from the viewpoint of the pathologist that Virchow[105] had actually hoped to draw attention to the cell as a chemical unit, as Klemperer[50] points out when he suggested the cell as the unit of disease

The disparity between the development of histological and biochemical information separated chemical constitution from the microscopic appearances To the student of disease it appeared frequently that the biochemists and the histologists were talking about two different structures For example the biochemist would talk about nucleo proteins and the histologists would speak about the nucleus The biochemists might mention that one of the types of nucleo protein would be found in the nuclei but not many biochemists would be able to recognize the nucleus under the microscope Contrariwise the histologists might mention nucleo protein in a discussion of nucleus and cytoplasm but it was apparent that not many histologists would recognize the chemical structure of the two types of nucleo protein This gap is being bridged by the renascent science of histochemistry

While the development of a science has to have a great many people contributing to it there can usually be several figures pointed out as being particularly influential In histochemistry the contributions of Raspail [87] of Ehrlich [30] and of Lison[8] appear particularly noteworthy The selection of these individuals does not imply that many other workers were not active in the field and did not contribute important material Actually the field has enjoyed the services of a great many scientists Contributions have come from all of the biological sciences Botany Zoology Histology Embryology and others as well as Pathology have represented fields in which histochemistry has been developed and found application Actually the real and potential contribution of the pathologists to histochemistry is very great as it was in the development of cytology and histology

The interest of the pathologist in the cells and tissues and the volume of tissues and cells which the pathologists study are both very great It will be recalled that Altmann was responsible for the isolation of nucleic acids from the nuclein of Miescher as well as contributing the cytological techniques for mitochondria and actually the description of the mitochondria themselves Altmann s fame was revived recently in the Altmann Gersh freezing drying apparatus now in such popular use in histochemical laboratories The names of pathologists who contributed to the early development of histology and cytology are too numerous to repeat but among them Benda Cajal Masson Unna Virchow and Policard should be mentioned Each of these students of disease attempted to learn something about the chemistry of the cell Chemical methods had not developed to the point at which their application to cellular constituents was possible Advance in chemistry has made histochemistry possible within recent years

Raspail was a versatile Frenchman who lived between the years 1794 and 1878. He was physician, botanist, chemist and strangely enough a politician of distinctly revolutionary tendencies. In this multiplicity of activities including violent politics as well as his original medical education he resembles the great figure of the French Revolution, Marat. Unlike Marat he died peacefully, although a number of prison sentences, exile and a flight to escape them are interspersed in the career of Raspail. The man has now reached a certain respectability and the Boulevard Raspail near the Sorbonne in Paris testifies to this.

Raspail is important in two ways. He seems to have been one of the first to have demonstrated chemical constituents in tissues. Moreover, he appreciated the importance of the demonstration. Colin and Declaubry had found in 1814 that iodine would stain starch granules. Caventou used this method in 1826. These are, however, isolated contributions while those of Raspail are numerous. He not only used the iodine method in 1825 for studying starch granules but also a method which is now used for tryptophane, a method for protein making use of nitric acid—the xanthroproteic reaction—tested the pH of the cytoplasm with an indicator dye and in general succeeded in his hope of "carrying the chemical laboratory to the microscopic stage." It is not too much to say with Baker[3] and Pearse[8?] that Raspail was the founder of histochemistry. Certainly the concept of histochemistry was first clearly stated by him. Above all he contributed valuable techniques, some of which are now in use.

The next figure which appears important on the scene of histochemistry is that of Ehrlich. The contributions of Ehrlich to medical and chemical sciences are almost too numerous to name. Specially noteworthy, however, are the introduction of arsphenamine for the treatment of syphilis and indeed the whole concept of chemical materials possibly poisonous to bacteria. Immunity still shows many of the marks of Ehrlich's contributions. Ehrlich in 1883 used iodine to stain glycogen in the tissues. In histochemistry Ehrlich's greatest contribution appears to have been the realization that enzyme could be demonstrated visually in the body tissues. Ehrlich injected a naphthol and paraphenylenediamine into animals. The blue color formed at sites of oxidase activity. Intellectually, therefore, Ehrlich is the ancestor and the one responsible for the many histochemical methods for enzymes which we have today. These include such valuable methods as those for the phosphatases[38,39] for cholinesterase[40] and for many other esterases. The method of Ehrlich is still used for cytochrome oxidase.[31]

The third figure that dominates the histochemical field is that of Lison. The studies of this contemporary Belgian scientist are many. Perhaps the most important is the organization of the material and concepts of histochemistry into his *Histochimie Animale*. This great contribution put histochemistry upon firm grounds, perhaps for the first time. Lison pointed out many of the basic criteria and desiderata of histochemistry. One of the basic ideas which is still worth considering from time to time is that histochemistry must be good histology as well as good chemistry. This is sometimes overlooked.

Worthwhile techniques are listed in Table III. The pathologist will recognize methods for iron and fat which are still in use.

Having gone along this far in our discussion of histochemistry and the histochemical approach it might be worthwhile to consider some definitions of terms. Histochemistry means literally the study of the chemical constitution of the tissues. By usage it includes cytochemistry, the study of the chemical constitution

of the cells Separation of the subject into histo and cytochemistry seems academic In this discussion whenever the term histochemistry is used it will include the study of the chemistry of the cell

TABLE III —HISTOCHEMICAL TECHNIQUES

Materials	*Methods*	*Authors*
Lipoid	Sudan II III	Daddi[24]
Lipids	Sudan Black	Lison[62]
Phospholipid	Chrome Hematoxylin Pyridine extraction	Baker[5]
Cholesterol	Lieberman Burckhard	Schultz[93]
Acetal phosphatids	Plasmal	Feulgen[33]
Ketosteroids	Phenylhydrazine	Bennet[8]
Steroids } Carotenoids }	Nile Blue Sulphate—Red stain	Cain[16]
Carbohydrate	Periodic Acid Schiff s (PAS) + acetylation	McManus & Cason[?]
Acid carbohydrate	Metachromasia + hyaluronidase + PAS	Penney & Balfour[83]
Glycogen	PAS + diastase	Lillie & Greco[69]
Protein	Diazonium	Danielli[2]
Arginine	Sakaguchi—Oxine	Warren & McManus[?]
Desoxypentose nucleoprotein	HCl—Schiff s	Feulgen[32]
Pentose nucleoprotein	Basophilia Ribonuclease	Stowell[?]
Alkaline phosphatase	Glycerophosphate calcium cobalt	Gomori[38]
Acid phosphatase	Glycerophosphate calcium lead	Gomori[39]
Dopa oxidase	Dihydroxyphenylalanine	Laidlaw[52]
Cytochrome oxidase	Nadi + Azide	Moog[9]
Choline esterase	Myristoyl choline	Gomori[40]
Ferric iron	Prussian Blue (Perls Stain)	Bunting[1]
Vitamin A	Fluorescence Microscopy	Popper[?]
Vitamin C	Silver Reduction	Bourne[13]
5 Nucleotidase	5 Nucleotide calcium cobalt	McManus Lupton & Harden[?]

PART I—TECHNIQUES

The techniques used in histochemistry are numerous They utilize the findings of chemical and physical techniques of many sorts They may be tabulated as follows

(1) Chemical reactions on tissue sections Example The PAS reaction for 1 2 glycols including carbohydrates[65]

(2) Chemical reactions produced by tissues and cells Example Enzyme demonstrations in tissues such as alkaline phosphatase[38] or cytochrome oxidase[31]

(3) Selective affinity of dye to tissue constituent Example The staining of fat with the Sudan dyes[?]

(4) The use of selective solubility in appropriate solvents to remove some tissue constituent [49]

(5) The action of enzymes on tissue sections It is considered that the enzyme will attack the appropriate substrate [37] Material removed by enzyme action is taken to be the chemical constitution of the appropriate substrate for this particular enzyme

(6) The use of radiation of different characters Ultraviolet absorption methods for the nucleo proteins[19] and the fluorescent microscopy [66] of tissues are examples of radiation

(7) Microincineration This method introduced by Policard [8] has found great use in the hands of many biologists It consists essentially in the gradual heating of a tissue section to a temperature at which all organic materials will volatilize, leaving the inorganic materials as a sort of skeleton of the cells and tissue The

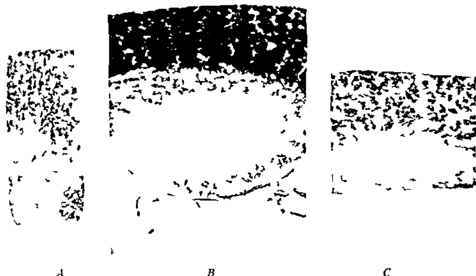


FIG 34 —*Alkaline phospatase on sections* A Crush failure B Normal kidney C Acute hypertension (McManus Medical Diseases of the Kidney)

further study of the remnants of the section covered with glycerin and a cover slip is best accomplished with dark field illumination It is difficult to identify the materials left in the ash

The chemical reactions in tissues would be the desirable method to study the morphology of tissue constituents This method has the disadvantage that static or fixed components only are studied For example the nucleus is generally studied by the Feulgen[3] reaction Only over wide ranges can the difference in the amount of nucleo proteins demonstrable by the Feulgen method be compared

The greatest care must be exercised in interpreting the chemical reactions produced on the tissue sections The repetition of the reaction in the test tube must be considered essential It should be demonstrated that the limits of sensitivity of the reaction conform to the limits of concentration of the material in the tissues It is by the chemical reactions on the tissues that the greatest or

Fig 35 —Above PAS reaction coloring mu in globules and brush borders human jejunum Below PAS stain human kidney glomerulus and tubules (McManus courtesy of Amer Jour Pathology)

at least most spectacular progress has been made It has not always been possible to present immediately the exact chemical bases for the chemical reactions but these have usually appeared in time

The chemical reactions produced by the tissues are numerous and increasing in number This is fortunate in many ways principally because one prefers to think of cells and tissues as being in a dynamic state If one were able to demonstrate only the fixed quantities of materials although they would have some morphological value the dynamic chemical information would be less

The chemical reaction produced by the tissue sections or cells consist generally in the exposure of the tissue or cells to a substrate which produces a colored or colorable compound For example in a demonstration of alkaline phosphatase there is one method[7] which produces a colored compound by the enzyme breaking up a soluble substrate and the product of the enzyme activity is deposited as an insoluble compound at the site of enzyme activity Alternatively in the demonstration of the alkaline phosphatase[33] there is the splitting of glycerophosphates by the enzyme in the tissue section The phosphate is rendered immediately insoluble by calcium present in the substrate solution Calcium is replaced by a cobalt wash following the electromotive series The cobalt is in turn colored black as the sulfide by yellow ammonium sulfide The black cobalt sulfide is presumably present at the sites of alkaline phosphatase activity

This technique of enzyme demonstration in tissues is susceptible to almost indefinite utilization The method of approach is limited by the necessity that the enzyme itself must not be diffused and that the compound produced by the action of the enzyme on the substrate must not be soluble Minor degrees of solubility have frequently produced erroneous interpretations Due to the many vagaries of technique it might be emphasized that comparison of histochemical results in this type of reaction are much better founded on a present or absent state rather than on a matter of degree It is fairly safe to say that when an enzyme should be demonstrated in large amounts in tissues and it is absent that there is something abnormal particularly when the enzyme can be demonstrated in normal quantities in adjacent tissue It is much less safe to say that the enzyme is decreased in quantity because such decreases may be the result of some condition of tissue handling Comparisons should be made only on tissues with duplicate handling

The prime example of the selective affinity of a dye for some tissue constituent is seen in the utilization of the alcohol and fat soluble Sudan dyes for the coloring of fat Frozen sections have ordinarily to be used but the solubility of the fat depends to a degree upon its association with other tissue components Many fats are actually not completely alcohol or even xylol soluble The chemical basis for Sudanophilia is not completely understood Apparently it depends upon the selective solubility of the Sudan dye in triglyceride fat possibly more complex fat in preference to the alcohol in which the dye is dissolved Actually there are a number of tissue constituents which do not appear to contain fat but which are colored by the Sudan dyes For example Sudan black[53] will color many mucins The mucins contain glycols The ordinary triglyceride fat is a fatty acid ester of glycerol The PAS reaction[65] of 1 2 glycols with fat has not yet been possible The alcoholic PAS method to be described later which was elaborated by Mowry and Longley[80] may make this possible If a chemical reaction which was specific for the triglyceride and other lipoids could be found it would be preferable to the

preferential solubility methods such as are used with the Sudan dye Until this technique is prepared it is necessary to utilize the Sudan dyes, keeping some mental reservations on their specificity

The use of solvents for removing materials from tissue sections and the use of enzymes on tissues are both limited by the one factor *i e*, the compound nature of tissue constituents Physically as well as chemically compounded mixtures of several different types of chemical materials occur in tissues The solubility of these mixtures in enzymes or in solvents is unpredictable The chemical impurity of tissue constituents is matched in regard to the enzymes by the impurity of the *enzyme preparations The naturally occurring enzymes attack a variety of* substrates For example, commercial pectinase has a protein active component as well as a carbohydrate active component It is therefore, impossible to draw too strict conclusions about enzyme specificity unless pure enzymes, preferably crystalized and electophorectically homogenous are used [71] Even with crystalline enzymes however there does appear to be the possibility that more than one type of substrate can be attacked [9]

The use of radiation of different wave lengths for the chemical analysis of tissues is a development of rather recent years While contributing a very great deal to the histochemical information the methods using radiation with a very few exceptions are yet so complex that the application in an ordinary laboratory of Pathology is not yet possible The only method of this sort in common use in laboratories of Pathology are the nicol prisms familiar to most pathologists for the identification of cholesterol The nicol prisms put light on one plane Material in sections which is frequently cholesterol is bi refrigent It is to be noted at this point that bi refrigence is a property of crystals rather than any particular type of crystal [6] The identification of cholesterol by bi refrigence in the nicol prism apparatus will be erroneous very frequently If there are other auxillary methods of identification such as some of the chemical reactions for sterols and perhaps even Sudan coloring a bi refrigent material probably is cholesterol but it need not be

Microincineration will be discussed no more than it has been above

This brief review of the principles of the histochemical identification of tissue has been presented as a preliminary to the study of a number of the classes of the chemical constituents of tissues These will be presented as critical comments on methods for carbohydrates fats and proteins Whenever possible the application of the methods to pathology will be mentioned

As in most reviews there will be more information or proposed information contradicted than actually presented That is necessary at the earlier stages of the development of a scientific discipline One of the first things that has to be done in the construction of a building whether it is a house or a *what not is the* clearing of the site This same situation holds true in the application of histo chemistry to pathology Many false conclusions have been presented The identification of blue staining granular material in tissues as calcium in the ordinary hematoxylin and eosin section is a prime example of this [17] Calcium very frequently does color blue with hematoxylin but so does nuclear material inspissated mucin and many other materials The identification of calcium in tissues is difficult The preliminary microincineration of the tissues or tissue section is to be desired By high temperature combustion of the tissue sections in a muffle furnace all organic material will be volatilized The crystalline material remaining

is of the inorganic variety and this does include calcium Even at this point the defining of the material as calcium is difficult

Carbohydrates

The carbohydrates of animal tissues are beginning to assume some of the importance which has been held for a very considerable time by the carbohydrates of plants Many, if not all animal tissues and cells appear to contain carbohydrate The older energy storage concept of glycogen has been reinforced by recent evidence Structural, lubricating and supporting roles are appearing for animal carbohydrates These newer data combine to expand our knowledge of the carbohydrates They produce a considerable revision of our ideas of normal and diseased cells and tissues from the twin viewpoints of structure and function

The new data upon the animal carbohydrates have come from two main sources The one and probably the more precise is in the biochemical studies of Feulgen,[3] Levene[94] Stacey[97] and many others The element of precision in these studies consists in the chemical formulation of the carbohydrates whenever the material under study existed in tissues in sufficient quantity to permit test tube analysis The other larger and more fruitful source of information is the application of chemical methods to tissue sections—the renascent study of histochemistry Admittedly less precise chemically, and limited in their application by the minute quantities of material which exist in the complicated cellular and tissue structure the data about carbohydrates furnished by histochemistry have been immense

The State of Carbohydrates in Tissues —Normal body constituents consist usually of mixtures or compounds of several classes of compounds *e g*, lipoproteins glycoproteins and glycolipids This complexity of composition is maintained at a structural level—nucleus and mitochondria—as well as in the molecular level—*e g* nucleoprotein Pure substances in the tissues appear to be rare Many if not all proteins contain small quantities of carbohydrate and in many proteins the carbohydrate content is sizable It may even be that the pure substances—fats carbohydrates and proteins—represent analytical values without exact counterpart in nature

The metabolic interrelationships of fat, carbohydrate and protein are obvious at the two carbon atom level Depending upon the ratio of these three constituents fat protein or carbohydrate material taken in as nourishment may transform into one of the other two materials *e g* the fat to protein or sugar

The carbohydrates of high molecular weight are classified by Stacey[97] as follows

Polysaccharides—no protein component glycogen

Mucopolysaccharides—small protein component mucins heparin chondroitin sulfuric acid

Mucoproteins—considerable protein component hormones

While the polysaccharides are taken to contain no protein the ability to fix glycogen in tissue with protein fixatives such as picric acid makes it probable that these materials have a colloidal relationship with protein if no other

The mucopolysaccharides comprise the best known group of the complex carbohydrates They include the materials in the ground substances of the connective tissues and cartilage the mucins etc

The mucoproteins include the hormones thyreoglobulin and the anterior pituitary follicle stimulating and lutein stimulating hormones The mucolipids are less well known but include many materials potentially important

In summary, from the chemical viewpoint there are carbohydrates in the tissues of animals in a form combined physically with protein as well as compounds in chemical combination with proteins The latter exist as chiefly carbohydrate or chiefly protein materials The individual carbohydrate building blocks of these polysaccharides mucopolysaccharides and mucoproteins can be taken to be present in tissues at one or another stage in metabolism

The Preservation of Tissue Carbohydrates for Demonstration in Histochemistry —Carbohydrates may be present in tissue without finding their way into sections for study Pure carbohydrates are water soluble especially those of low molecular weight The routine histological and cytological fixatives contain water as do the dilute alcohols which begin dehydration Aqueous solutions of stains and chemicals are used in the demonstration of carbohydrates It would be expected that most or all of the carbohydrates would be lost from sections especially those without protein components

Fortunately this is not the case As previously mentioned even the polysaccharides appear to have a physical combination with protein Some glycogens in tissue can be preserved with aqueous fixatives such as Zenker s fluid or Helly's fluid (Zenker s with formol) or even with such solutions as neutral formalin This has been repeatedly described recently by McManus and Findley[70] concerning the glycogen of the human cervix uteri Fixatives containing picric acid especially Bouin s fluid are especially *good for glycogen in tissues* This has been emphasized by Lison[6] by Pasteels and Leonard[81] and recently by Lillie[58] It is an overlooked fact which invalidates many conclusions drawn from the pure polysaccharides rather than from their state in the tissues

If the mucoproteins and mucopolysaccharides contain sufficient protein any efficient protein fixatives will fix the entire complex This is especially true of the mucoproteins which are well fixed by Zenker s Helly s Regauds Maximov s or Bouin s solutions For the mucopolysaccharides with small protein content it may be necessary to use fixatives such as lead sub acetate (Holmgren s Fixative[4]) This was recommended by Dempsey and Wislocki[28] who point out its distinct shortcomings as a cellular fixative A recent suggestion that the osmium tetroxide fixatives[77] may be valuable for the mucopolysaccharides and the polysaccharides needs further confirmation and study Some preliminary observations by McManus and Lupton (unpublished results) are summarized here

The use of osmium tetroxide as a carbohydrate fixative has proceeded only a short while In a few instances in the experimental animal (rabbits and rats) it has seemed to preserve these tissue structures and carbohydrates in fine fashion We have used it in a solution made up as follows

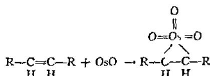
Normal saline	50 cc
Bichloride of Mercury	10 gm
Osmium tetroxide	1 gm

The bichloride and osmium are dissolved in the order given The fixative is kept in a sealed brown bottle and is used in small vials which are sealed

The osmium fixative suffers from the customary handicap of poor penetration so that very small portions of tissue must be used It has seemed that with small and thin portions of tissue adequate fixation has occurred when the central por

tions of the tissue are yellow rather than black. This has occurred in one to six hours. After this period of fixation the tissue is washed and dehydrated rapidly by an acetone method or more slowly by an alcohol xylol procedure. The tissue is embedded in paraffin and sections can be cut readily at 4 to 6 microns.

The glycogen of cartilage cells is well fixed after one hour and superbly fixed after four hours. The longer fixation has the disadvantage that other tissue carbohydrates (mucin, etc.) are decreased in quantity, perhaps by overoxidation. The osmium tetroxide blackens some materials such as myelin sheath which can in turn be oxidized by periodic acid and colored with Schiff's reagent. It is possible that this material in the myelin sheath was originally carbohydrate. It is also possible that an unsaturated double bond has been oxidized by the osmium which is attached to it in its reduced condition, *i.e.*



The subsequent oxidation by periodic acid of the osmium compound might produce aldehyde which is colored by Schiff's reagent. The other possibility is the initial vigorous oxidation of a double bond[19] to produce a 1,2 glycol which is then susceptible to oxidation by periodic acid.

An important sequence of the osmium tetroxide experiments is the realization that periodic acid may produce a coloring where no carbohydrate is present.

Freezing drying methods find their most valuable application in the study of the polysaccharides and mucopolysaccharides. It is possible to preserve these carbohydrates in their proper cellular and histological position in most instances with the Altmann Gersh method.[38] The difficulties of delay and complicated expensive apparatus restrict the use of this valuable technique to research laboratories. Inevitable simplification will make the method more widely usable. In the meantime fixation in picric acid alcohol following rapid cooling has been recommended by Lison.[63]

Once successful fixation has been accomplished the processes of dehydration, clearing and paraffin embedding have little danger for the carbohydrate. Celloidin embedding is slower but at least as good as paraffin embedding. The preservation of carbohydrate in tissue demands the optimum handling at every stage including the hydration following sectioning. Lillie recommends a coating of the section with celloidin before it is brought to water preliminary to the demonstration of the carbohydrate. This procedure is useful in the study of human liver glycogen. It is not necessary for other glycogens, *e.g.*, cervix uteri or for most mucopolysaccharides and mucoproteins.

Methods for the Demonstration of Carbohydrates in Tissue Sections. — If there are carbohydrates in tissues and they are adequately preserved into the tissue sections their demonstration is unsatisfactory unless a specific reaction is used. For example, it is of little value to preserve the carbohydrate into tissue sections if they are then demonstrated by one of the carmine dyes which color non carbohydrate materials and whose chemical basis for staining is unknown.

In summary, from the chemical viewpoint there are carbohydrates in the tissues of animals in a form combined physically with protein as well as compounds in chemical combination with proteins. The latter exist as chiefly carbohydrate or chiefly protein materials. The individual carbohydrate building blocks of these polysaccharides, mucopolysaccharides and mucoproteins can be taken to be present in tissues at one or another stage in metabolism.

The Preservation of Tissue Carbohydrates for Demonstration in Histochemistry.—Carbohydrates may be present in tissue without finding their way into sections for study. *Pure carbohydrates are water soluble*, especially those of low molecular weight. The routine histological and cytological fixatives contain water as do the dilute alcohols which begin dehydration. Aqueous solutions of stains and chemicals are used in the demonstration of carbohydrates. It would be expected that most or all of the carbohydrates would be lost from sections, especially those without protein components.

Fortunately this is not the case. As previously mentioned, even the polysaccharides appear to have a physical combination with protein. Some glycogens in tissue can be preserved with aqueous fixatives such as Zenker's fluid or Helly's fluid (Zenker's with formol) or even with such solutions as neutral formalin. This has been repeatedly described recently by McManus and Findley[70] concerning the glycogen of the human cervix uteri. Fixatives containing picric acid, especially Bouin's fluid, are especially good for glycogen in tissues. This has been emphasized by Lison,[6] by Pasteels and Leonard[81] and recently by Lillie.[58] It is an overlooked fact which invalidates many conclusions drawn from the pure polysaccharides rather than from their state in the tissues.

If the mucoproteins and mucopolysaccharides contain sufficient protein, any efficient protein fixatives will fix the entire complex. This is especially true of the mucoproteins which are well fixed by Zenker's, Helly's, Regaud's, Maximov's or Bouin's solutions. *For the mucopolysaccharides with small protein content* it may be necessary to use fixatives such as lead sub acetate (Holmgren's Fixative[4]). This was recommended by Dempsey and Wislocki[8] who point out its distinct shortcomings as a cellular fixative. A recent suggestion that the osmium tetroxide fixatives may be valuable for the mucopolysaccharides and the polysaccharides needs further confirmation and study. Some preliminary observations by *McManus and Lupton (unpublished results) are summarized here.*

The use of osmium tetroxide as a carbohydrate fixative has proceeded only a short while. In a few instances in the experimental animal (rabbits and rats) it has seemed to preserve these tissue structures and carbohydrates in fine fashion. We have used it in a solution made up as follows:

Normal saline	50 cc
Bichloride of Mercury	10 gm
Osmium tetroxide	1 gm

The bichloride and osmium are dissolved in the order given. The fixative is kept in a sealed brown bottle and is used in small vials which are sealed.

The osmium fixative suffers from the customary handicap of poor penetration so that very small portions of tissue must be used. It has seemed that with small and thin portions of tissue adequate fixation has occurred when the central por-

phosphate and Wislocki[107] and Meyer[76] have shown metachromasia in hyaluronic acid and in ribonucleic acid It may be stated fairly that the chemical basis for metachromatic staining is not certain although metachromasia is a property possessed by many carbohydrates particularly those of high molecular weight and acid in reaction

Methods with Carmine —It is not clear that the carmine stains for carbohydrates *e g* Best s Carmine Stain for glycogen[11] Mayer s Mucicarmine Stain for mucin[66] are not based upon some poorly understood chemical affinity At present time these are empirical methods which are not specific or trustworthy Best s Carmine stain for glycogen frequently colors mucus and mast cell granules Mayer s mucicarmine may color glycogen It can be shown (vide infra) that other methods for demonstrating glycogen show more material than Best s carmine Under these circumstances—Best s Carmine is not specific for glycogen and does not demonstrate all the glycogen—the method should be allowed to disappear from practice

The mucihematein stain of Mayer[66] has been popular in the past It stains mucin a clear blue and shows connective tissue mucin better than epithelial mucin Some of the mucins —*e g* , stomach— are not shown at all It is interesting that both mucicarmine and mucihematein stains contain aluminum chloride These stains might be of some value if their chemical bases were understood

Congo Red —While amyloid does contain some carbohydrate it is only possible that its selective coloration with Congo Red depends upon carbohydrate However there is such a selectivity of Congo Red for amyloid that it is probable that there is a chemical basis Congo Red will stain amyloid vitally if injected intravenously and in sections In the test tube a faintly alkaline solution of Congo Red can be used to color fibrin so that tryptic digestion may be followed[4] This makes it possible that its combination is not with protein in fibrin and it may not be in amyloid While useful histologically Congo Red has no histochemical value since in sections it will color fibrin fibrinoid and necrotic hyaline as well as amyloid

Oxidation Methods —The most precise of the methods for the demonstration of carbohydrates are those which are based upon oxidation Naturally the most specific of these would be the reaction whose chemical basis is most limited and best known It will be shown that periodic acid oxidation fits these conditions

The demonstration of carbohydrates by oxidation methods dates from the discovery by Bauer[7] that glycogen will re color Schiff s reagent after chromic acid oxidation The method was appreciated by Lison[6] and C M Bensley[9] as far superior to Best s Carmine for the demonstration of glycogen Wallraf and Bechert[103] showed that Bauer s method colored a number of non glycogen carbohydrates including the ground substance of cartilage and mucin in sections fixed by various methods or by changing the conditions of oxidation slightly

Chromic acid has the defect that it is a broad spectrum oxidant attacking materials other than carbohydrates Similar criticism can be made of potassium permanganate (Casella[18]) and hydrogen peroxide (Lillie[8]) as oxidizing agents This defect is not shared by periodic acid

An aqueous solution of periodic acid (meta periodic acid HIO_4 or H_5IO_6) has a spectacular specificity for 1 2 glycols[6] The product of oxidation is aldehyde and in histochemistry the aldehyde is colored with Schiff s reagent The whole reaction may be represented

The earlier studies of tissue carbohydrates utilized a wide variety of empirical coloring methods The first of these seemed to have been iodine whose affinity for starch made it a logical material to try in the coloration of animal starch or glycogen [10] There is no record of the application to animal tissues of the copper reduction method of Sachs used in botany [89] Schulte s Chlor Zinc Iodine[94] is not useful in the study of human tissues despite its value for plant carbohydrates (unpublished studies) Ruthenium red, the dye used for pectin materials in plants, was used by Heidenhain to study the ground substance of the ligamentum nuchae I know of no other study of animal carbohydrate with this dye It has seemed not useful in some preliminary studies of my own (unpublished)

Metachromatic staining is a property possessed by some carbohydrates, notably mucin Some basic dyes (thionine, methyl violet, sparanin etc) impart a color to mucin, moist cell granules, ground substances of cartilage, etc which is different from that of the nuclear and cytoplasmic stain Ehrlich[30] first observed this phenomena and introduced the term metachromasia The phenomenon essentially consists of two parts (1) the dye which stains, and (2) the material which is stained Too many of the considerations of the process of metachromatic staining do not differentiate these features They will be considered here separately and in order

(1) *Metachromatic Dyes* —The best known of these are Thionine, Crystal Violet and Methyl violet *Toluidine blue, Safranine Polychrome Methylene Blue* and Iodine Green The latter two are definitely not metachromatic dyes since they are dye mixtures and the different staining effects are due to one or another dye The other metachromatic dyes are those which have a different color in *basic and acid solutions They represent several different compounds which have* these features in common

(2) *Metachromatic Materials* —Under ordinary fixations the following materials are usually metachromatic the ground substance of cartilage and, where preserved *of connective tissue* Wharton s Jelly of the umbilical cord, mast cell granules and blood basophiles mucin of many or most mucus producing epithelial glands and cysts amyloid infiltrations fibrinoid necrosis occasionally fibrin and colloid droplets These include most of the hyaline materials of tissues

Bignardi[1] *has pointed out that glycogen becomes metachromatic after oxidation* Others describe all tissue constituents becoming metachromatic after prolonged oxidation Paneth cells possess metachromasia after oxidation The negri or tigroid bodies of nerve cells are sometimes metachromatic as are bodies in liver cells and the placental syncitium The studies of Lillie[60] have shown a wide difference in the metachromasia of gastrointestinal mucins varying with the dye the species of animal and with the level of the intestinal tract I have found that the mucin of mucinous intestinal tumors may be metachromatic with dyes which do not color the corresponding normal mucin metachromatically and vice versa Follis[31] has lately shown metachromasia in human skin collagen after enzyme action

The chemical basis for metachromatic staining is not certain It was thought by Ehrlich[30] that metachromasia gave some hint about chemical structure Michaelis[77] believed that the dyes were tautomeric and were altered by the state of the material in the tissue Lison[6?] had suggested that metachromatic staining was a property of sulfate esters of carbohydrate polysaccharides This now appears unlikely since Wiame[108] has shown metachromasia in solutions of hexameta

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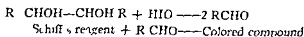
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R CHOH—CHOH R + HIO ——2 RCHO
Schiff's reagent + R CHO——*Colored compound*

Several features are worth study in this reaction including substituted forms of glycols susceptible of periodic acid oxidation, other materials oxidizable by periodic acid and the features of Schiff's reagent

The mono amino substituents and the alkylamine mono substituents of 1 2 glycols are oxidized by periodic acid[43] This occurs most readily at an alkaline pH but may occur at an acid pH over a prolonged period of time The rate rather than the specificity is changed by alteration in pH

Other materials, including phenols may be oxidized by periodic acid The oxidation of phenols by periodic acid is accompanied by yellow and red colorations according to Pennington and Ritter[81] This should be studied in sections when an object is thought to be colored by a phenol oxidization effect Only resorcinol and phloroglucinol out of 18 phenols tested did not give a yellow or red color No definite end products of periodic acid oxidation of phenols were identified by Pennington and Ritter Although these authors did not specify the absence of aldehyde it may be presumed

Schiff's reagent is now nearly one hundred years in use as a reagent for aldehydes[9] It is customary from time to time to have it indicated that Schiff's reagent a bisulfite compound with basic fuchsin, can be recolored by non aldehyde materials[6] I know of no instance in which satisfactory proof was given that the material concerned was actually aldehyde free with the exception of the bromine whose presence is unlikely in tissues

There is a considerable amount of variation in the preparation of Schiff's reagent * It is most readily prepared by bubbling SO_2 into an aqueous solution of basic fuchsin Other methods including the one in personal use produce the SO_2 in the solution slowly by the inter action of acid on bisulfite The preparation of the reagent takes longer overnight in place of a few hours but the final product has certain advantages Schiff's reagent is essentially fuchsin bisulfite in a solution of sulfurous acid The excess of sulfurous acid is guaranteed by the slower method of preparing the Schiff's reagent

Removal of any remaining color from the Schiff's reagent is completed in some techniques by filtering it through powdered charcoal The final solution is straw colored perhaps with a faint pink shade The removal of color by charcoal is associated with the removal of some of the SO_2 and this should be compensated by the addition of more SO_2 This latter process is certain to decrease the sensitivity of the Schiff's reagent but will assure the destruction of periodate which might conceivable be attached to the section

The original publication describing the use of periodic acid gave directions for the use of an aqueous solution of 1 per cent HIO_4[84] After two minutes oxidation the section was rinsed in distilled water and placed in Schiff's reagent The customary rinsings in sulfurous acid followed and the section was immediately dehydrated cleared and mounted in balsam The later method[69] described the oxidation lengthened to five minutes and added a rinsing in running tap water after the sulfurous acid rinses This returned a good bit more color to the section

* See Arzac's method for demonstrating aldehyde forming the Schiff reagent on the slide (p 74)

Lillie in 1947 [7] independently described a periodic acid method for tissues producing the acid by a mixture of sodium periodate and 0 5 per cent Aqueous concentrated Nitric acid The further steps were similar to the original description, as were the results

During the years in which the descriptions of McManus and Lillie using periodic acid oxidation as a method for carbohydrates appeared the independent discovery of Hotchkiss[46] dating from 1945 was proceeding along similar lines Periodic acid was used as in the methods of Lillie and McManus but with the major difference that a reducing rinse was interposed between the periodic acid oxidation and the Schiff s reagent The reducing rinse was composed of an acidified thiosulphate solution It was provided to remove the possibility that periodate or iodate might combine with calcium or potassium which might be present in the tissues

Hotchkiss article placed the periodic acid Schiff s method for carbohydrates on a solid chemical basis While not realizing that all glycogen was not water soluble the study was probably the beginning of the histochemical investigation of the periodic acid methods The histochemical studies of both McManus and Lillie were more along histological lines in which they were quite productive By the introduction of the reducing rinse Hotchkiss considerably altered the histological picture produced in sections while improving it seemed the chemical validity of the methods For example mucin and glycogen color quite as brilliantly as ever with the Hotchkiss method but basement membrane reticulin etc are barely colored compared to the results of the method of either McManus or Lillie

The first explanation of this discrepancy arose in the demonstration that the reducing rinse would decrease the coloring of nuclei with Schiff s reagent if it were interposed into the Feulgen method just after the HCL hydrolysis [69] This has been repeatedly confirmed as has the decrease in final color by the reducing rinse after periodic acid oxidation It seems at the present time that the bisulfite or thiosulfate is binding some of the aldehyde groups produced by the periodic acid oxidation (McManus and Pigman, unpublished) since the same effect can be produced by aqueous thiosulfate

The danger of periodate recoloring Schiff s reagent is not a real one under the conditions used in histochemistry It can be shown (McManus and Hoch Ligeti[74]) that the periodic acid and Schiff s reagent can be mixed in the test tube without a permanent precipitate occurring until the periodic acid makes up more than one fifth of the volume of the Schiff s reagent in the mixture The precipate when formed is yellow or straw colored rather than the purple of the aldehyde bisulfite fuchsin The precipitate between periodic acid and Schiff s reagent can be re dissolved in an excess of Schiff s reagent These observations suggest that any periodic acid from periodic acid oxidation which may be attached to the section can be dissolved in Schiff s reagent without a reducing agent being necessary

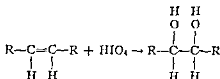
The difficulty of periodate and Schiff s reagent combining with calcium to form a colored compound could be serious in histochemistry Any combination of calcium chloride periodic acid and Schiff s reagent which we have tried has no color *in vitro* provided the proportions of reagents are 1 1 5 as in the other *in vitro* tests and that the Schiff s reagent is fresh

As far as we can make out chemically theoretically *in vitro* and on sections provided that relatively fresh Schiff s reagent is used a reducing rinse is not necessary after periodic acid oxidation and before Schiff s reagent The method

(the PAS method) appears specific for 1 2 glycols and their amine substituents

Mowry and Longley[90] have developed an *elegant modification of the PAS* method which makes use of alcoholic solutions throughout They point out that a number of water soluble carbohydrate materials can be preserved and demon strated by this method McManus and Graham have used this method for the PAS *positive materials of the human aortic media In all ages, the PAS* positive material presumably carbohydrate, is present in considerable quantities Glycogen is, however, less well shown by the alcoholic PAS method than by the aque ous PAS perhaps due to insolubility of reactive groups in the alcohol

A further complication is presented in the recent description by *Wollman*[108] of unsaturated fatty acids which give a positive coloration with Schiff s reagent after periodic acid oxidation The first stage in the oxidation of a double bond appears to be the formation of di hydroxyl compounds replacing the double bond and this 1 2 glycol is split to form aldehydes The reaction might be set out in the following diagram



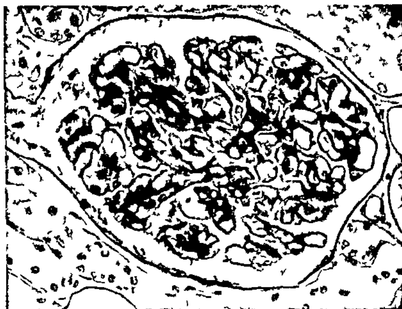
This eventuality or possible error can be overcome by the use of osmium tetroxide which settles out at sites of double bonds or by the acetylation method (pp 155)

Combinations of the PAS method with other histological and histochemical methods are possible A Feulgen Test may be done prior to the PAS allowing a nuclear stain which is about the same color as the PAS Fat may be colored with Sudan IV or Sudan black in frozen sections after the PAS method Hematoxylin as a counterstain is possible and Lillie recommends Light Green as a counterstain Iron may be stained after the PAS method The elastic tissue staining by Orcein is possible as is connective tissue staining by Mallory s Aniline Blue Orange G Before describing histochemical methods combining the PAS with the other techniques it is necessary to describe another reaction for carbohydrates

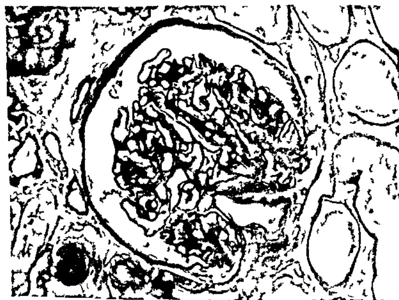
Hale[41] in 1946 described the affinity of acid mucopolysaccharides of tissue for dialyzed ferric iron After the tissue was soaked in a buffered solution of dialyzed iron it was well rinsed and the ferric iron was demonstrated by the familiar Prus sian Blue Method The result was a blue staining of many of the tissue structures which color with the PAS method *i e* mucin of some epithelia and of most con nective tissues reticulin etc The positive results vary from species to species but they are pretty consistent from slide to slide The chemical basis for the Hale stain does not seem to be as certain as the periodic acid methods It seems probable that any acid group is shown whether carbohydrate or not Combined with the periodic acid method the Hale stain seems to have considerable utility

Ritter and Oleson[88] have recently made such a combination Sections fixed in alcohol formalin are colored by the Hale method and then a PAS is done on them The reaction of certain structures which ordinarily color by the PAS method is blocked while others combine the PAS and Hale coloring or color with the PAS alone In personal studies it has been found best to color a corresponding section with the PAS method whenever the Ritter and Oleson stain (PAS Hale) is done This precludes the false interpretation of such structures as nuclei which may color

PLATE II



A Normal glomerulus of a human kidney colored with the Ritter and Oleson (50) method. The glomerular intercapillary space is colored red. The glomerular basement membrane over the capillaries is colored blue. Kodachrome × 250



B Human renal glomerulus as in *A* from a case of intercapillary glomerulosclerosis. The abnormal glomerular material is colored red and accentuates the intercapillary space. Kodachrome × 190

(From McManus, Lupton and Graham, courtesy of Anatomical Record.)

with the Hale method Actually any fixation is possible, Zenker formol being quite good McManus Lupton and Graham have described and illustrated the superior coloring of the human renal glomerulus by the Ritter & Oleson method (Plate II)[73]

Monne and Slautterback[78] have found that phenylhydrazine will color some but not all of the re active groups left after periodic acid oxidation which ordinarily attach to Schiff s reagent Varying the period of time in the phenylhydrazine changes the effect Many of the structures color with Schiff s reagent if the time in phenylhydrazine is short while a longer time in phenylhydrazine decreases the coloring with Schiff s This sort of study has been taken by the authors men tioned to indicate different carbohydrates in the tissues a conclusion not com pletely justified by the data presented The older reaction of Cretin [3] for glycogen which employed phenylhydrazine as a mordant on blocks of tissue or on sections is recalled Glycogen was then colored with Schiff s reagent according to Cretin Here then in Monne and Slauterback's method we have phenylhydrazine which will form colored compounds with some aldehydes and which will itself oxidize glycogen to aldehydes introduced between periodic acid oxidation and the Schiff s reagent This is certainly a complicated procedure whose chemical basis deserves considerable study

The proof of carbohydrate identity should not be allowed to rest solely upon a single reaction even one as specific as the PAS When adjuvant methods are possible the further definition of a tissue structure as carbohydrate should be attempted These must be chemical and include reactions on the slide as well as enzyme action

The most precise of these is the acetylation technique [7] This depends upon the fact that a mixture of acetic anhydride and pyridine will acetylate a tissue section Acetyl groups are attached to hydroxyl and amine groups as well as to carboxyl groups Brief saponification in weak alkali will remove the acetyl groups from hydroxyl radicles but leaves unchanged the acetylated amines The applica tion of acetylation to the study of tissue carbohydrate requires three parts (1) the PAS reaction on a tissue (2) acetylation of the tissue followed by the PAS reac tion and (3) acetylation of the tissue saponification and the PAS reaction A material which is positive in 1 and 3 and negative in 2 is probably carbohydrate For example the unsaturated lipoids are positive in all three the PAS reaction not being blocked by acetylation

The acetylation saponification method has shown certain features upon further study which deserve mention Various batches of acetic anhydride differ in their ability to acetylate This fact has been recognized a long time in carbohydrate chemistry and appears to depend upon the amount of unaltered acetic anhydride remaining in the solution Re distillation and purification may be required to obtain optimum acetylation With impure acetic anhydride the time which the section is left in acetic anhydride pyridine may need to be lengthened up to two or three hours Contrariwise leaving the sections in the saponifying solution for forty five minutes results in an inevitable loss of some sections from the slide Actually, ten minutes in 0 1M KOH will saponify the acetylated hydroxyl groups and save many sections

The preceding essentially consisted of a specific method to block the PAS reaction It is possible to identify the material produced by periodic acid oxida tion as aldehyde by the use of aldehyde reagents such as hydroxylamine The

water but can make emulsions with it The fats or lipids are usually separated into simple lipids—the alcohol esters of fatty acids— and complex lipids or lipins

Simple lipids include the triglycerides and the waxes The triglycerides are fatty acid esters of the carbohydrate glycerol Waxes are esters of fatty acids with non glycerol alcohols The fatty acids are of the stearic group—saturated oleic group one double bond linoleic group two double bonds linolenic group three double bonds etc In the triglycerides the fatty acids are of the same or different types Glycerol or glycerin is a trihydroxy alcohol The non glycerol alcohols found in the waxes are monohydroxyl or dihydroxyl

The complex lipids or lipins have been lately re classified by Thaunhauser and Schmidt[100] It is probable that the lipins are the more important fats in the human while in the lower animals and especially the invertebrates the triglycerides and waxes are more important Hilditch[44] has shown that climbing the evolutionary scale has been associated with a shortening of the fatty acid chain and an increase in saturation Even within the one species of fish the change from marine to fresh water habitat is associated with this type of change Likely the lipin to triglyceride ratio (complex lipid simple lipid ratio) increases as the evolutionary scale is climbed

The classification of Thaunhauser is as follows

(1) *Monoaminophosphatides* Fatty acid esters of a phosphorylated polyvalent alcohol combined with a nitrogen containing group Their ratio P N is 1 1 (Some monaminopho phatides contain other organic groups in addition to those already mentioned)

A Lecithins Phosphoric acid diesters of diglycerides and choline
Lysolecithins Phosphoric acid diesters of saturated monoglycerides and choline

B Cephalins All known cephalins contain their total nitrogen in form of a primary amino group (ethanolamine or serine)

a) Phosphatidyl ethanolamines
Phosphoric acid diesters of diglycerides and ethanolamine

b) Phosphatidyl serines
Hydrolysis products Fatty acids phosphoric acid polyvalent alcohols serine

(2) *Plasmalogens* (Acetalphosphatides) Phosphoric acid diesters of a higher fatty aldehyde acetal of glycerol and of ethanolamine

(3) *Inositol phosphatides* obtained from brain soybeans and bacteria The ring carbohydrate inositol in a complicated structure not completely known

(4) *Phosphatidic acids cardiolipins* Hydrolysis products Fatty acids polyvalent alcohols phosphoric acids (bound as a monoester)

(5) *Phosphatides of acid fast bacteria* Hydrolysis products Phosphoric acid polyhydroxy compounds (such as carbohydrates inositol) fatty acids with straight and branched chains

(6) *Diaminophosphatides* (Sphingomyelins) Acid amides of sphingosine with fatty acids (ceramides) in ester linkage with pho phorylcholine

(7) *Cerebrosides* Acid amides of fatty acids with sphingosine or dihydro sphingosine in glucosidic linkage with galactose or glucose

(8) *Gangliosides* Structure unknown hydrolysis products sphingosine neuraminic acid fatty acids and galactose or glucose

reaction is carried out as follows Duplicate sections are oxidized with periodic acid One of these is placed in distilled water, the other in hydroxylamine solution After an appropriate interval both are rinsed in distilled water and placed in Schiff s reagent for fifteen minutes The coloring present in the section which was left in distilled water and not present in the section left in hydroxylamine is clearly due to aldehyde formed by periodic acid oxidation, *i e* from 1,2 glycols probably carbohydrate

The hydroxylamine blockage of the Schiff's reagent may prove to be a useful method in the study of tissue carbohydrate I have had no experience with it It is likely that a combination of acetylation saponification and aldehyde blockage will give a pretty definite specificity for the PAS method in the study of tissue carbohydrates

The action of enzymes on tissues is one of the older methods for identifying tissue carbohydrates It has been shown, for example that saliva will remove glycogen from tissue sections [9] This action is thought due to salivary amylase Lillie and Greco[59] introduced malt diastase as more aesthetic than saliva for glycogen digestion but shortly thereafter reported the removal of pancreatic basophilia presumably due to ribonucleic acid by malt diastase solution [61] It is interesting that this cytoplasmic basophilic material not infrequently gives a positive PAS reaction questioning our structural concepts of ribonucleic acid This has received recent emphasis from test tube studies on ribonucleic acid by Allen

The introduction of the pectin group of enzymes promised to expand very considerably the utility of the enzyme on tissue studies [71] The action of pectin methyl esterase and pectinase are thoroughly studied and well known because of their use in the citrus fruit industry It has been found that the enzymatic activity of both pectinesterase and pectinase depend upon hydroxyl groups by the acetylation saponification techniques as does malt diastase [74] Pectinesterase increases the PAS coloration of tissue carbohydrates after any fixation while pectinase removes it after acetone fixation All these data appeared to fit into the scheme of our knowledge of tissue carbohydrates

Unfortunately it has not yet been possible to free the pectin group of enzymes from proteolytic activity The commercial source of these enzymes is the penicillium group of fungi especially penicillium Oryzae The enzymes are secreted apparently as a means of digesting potential food materials of plant origin and the organism makes no selection between proteolytic and saccharolytic enzymes nor can the laboratory to date This complexity of enzyme activity very definitely limits the possibility of the use of the pectin group of enzymes in histochemistry

Actually the same criticism may be raised against the use of saliva or malt diastase and the amylase (a and b) of malt B glucuronidase has no effect on tissue The hyaluronidases remove metachromasia from various tissue structures but do not remove their PAS positive material [28] Hyaluronidase has further been shown to act on chondroitin sulfuric acid as well as hyaluronic acid so that its specificity within the carbohydrates is in question [47 75]

Fats

Fats are loosely defined as a group of tissue constituents which are soluble in organic solvents of the benzene ether chloroform group They are insoluble in

which are resistant to decolorization by Wiegert's differentiating solution Other materials are colored by this method as well as the phospho lipids and Baker has introduced a solubility or extraction step which removes lipids from sections The test essentially consists of a performance of the test on one piece of tissue with hematoxylin and the performance of the test on another piece which has been through the lipid solvent The material which colors in the first section and which does not color in the second is in all probability phospho lipids Baker points out that all of the phospho lipids present in the tissue may not be stained by his method but it is made quite clear that materials which do stain with his method are in all probability phospho lipids

I know of no study of human tissues with Baker's method This badly needs to be done since the method has proven its value in animals In animals it shows mitochondria and the golgi element

The acetal phosphatides are of special interest in pathology because of their presence in the adrenal cortex The cortical hormones appear related in some at present ill-defined fashion with the acetal phosphatides Much further study is needed This should be one of the more profitable fields for the pathologist

The acetal phosphatides are demonstrated in frozen sections by the action of mercuric chloride The mild action of mercuric chloride which is supposed to be oxidizing or hydrolysing agent liberates aldehydes which are colored with Schiff's reagent It is important that brief fixation is used the acetal phosphatides being studied preferably within twenty four or forty eight hours The methods for the demonstration of this group of substance have been reviewed by Cain[16] and by Leblond[1]

Essentially the acetal phosphatides which are shown by the Schiff's reagent after mercuric chloride compose the plasmal and plasmalogen materials of Feulgen[33] Under these latter two terms a considerable literature has accumulated At the present time it seems preferable to group these materials under the heading of acetal phosphatides

Ketosteroids will give a positive reaction with the Schiff's reagent One of the future tasks of lipoid histochemistry is to find some satisfactory way of separating these materials The recent literature has been well summarized by Pearse[8] Some later studies appear in the proceedings of the second annual meeting of the Histochemical Society This field of acetal phosphatides and indeed the whole human adrenal cortex is a prime example of a research which could be entered into by a pathologist with great hopes of success

Cain in his review differentiates between the solid and liquid lipoids Solid lipoids are found in low animals particularly the invertebrates but I do not know of any lipoids which are solid at room temperature or at body temperature in the human It may be that some of the products of fat necrosis of the diabetic or the non diabetic varieties would fall into this group In this case it might be advantageous to raise the temperature of the staining solution to 38° C or higher

Cholesterol is practically impossible to define as such in tissues It has the property of bi refringence as previously described The sites from which cholesterol can be extracted are frequently colored by the Schultz modification[93] of the Liebermann Burckhard reaction[55] It is important in interpreting results that a positive result for sterols is given when the color appears as red and changes through blue to green Any one of these colors not changing is not a positive test as Lison and Cain point out Bennet's phenylhydrazine reaction[8] has been

The fatty acids in lipins have been studied The ether soluble lipins contain even chains of 16 to 22 carbon atoms Twenty four carbon atoms are found only in cerebrosides and sphingomyelin

The fatty acids in liver are much like those in the monoaminophosphatides The storage fats are unlike monoaminophosphatides in that 20 and 22c unsaturated acids are absent

The complex lipins are seen to be combinations of phosphoric acid and fatty acids with carbohydrates or at least polyvalent alcohols These dissimilar radicles in the molecules suggest a multiple activity for the complex lipins It is possible that portions of the molecule may alter their energy content—the phosphoric acid element especially—while the main molecular mass is still in a condition to perform a supportive or relatively subsidiary function This may be the basis for the occurrence of the complex lipins in active portions of cells—cellular particles the mithochondria and golgi element—and in special tissues such as nervous tissue

The classical method for the staining of fats in tissue makes use of an alcohol or an alcohol acetone solution of one of the Sudan dyes Herxheimer[43] recommends an acetone alcohol mixture for the solution of Sudan dye Sudan IV or Scarlet Red is the most commonly used of the Sudan dyes

Sudan Black was introduced by Lison[6] in 1936 With sections which compare the staining with Sudan IV and Sudan Black there are a number of structures which color with the Black dye which do not color with the Red one Generally the intensity of staining appears increased This latter apparent intensification of staining may be due to nothing more than the black color compared with the red one Myelin sheaths of the nervous system and various intracellular organized structures are colored with the Sudan Black although they remain uncolored with the red or scarlet Sudan dyes It can be shown in the test tube and it is highly probable in the sections that Sudan Black is coloring some of the complex lipids

Sudan Black has the advantage for pathology that non lipid materials such as mineral oil are not colored by it Mineral oil granulomas of the lung will color with Sudan IV but not with Sudan Black[6] This is a useful differentiation in the study of 'lipoid' pneumonias and granulomas

The amount of complex lipids colored in frozen sections depends upon fixation A cobalt calcium formalin mixture[67] and a cadmium calcium formalin mixture[4] have definite advantages over the ordinary formol saline However, the ordinary formalin saline can be used if too long fixation is not permitted that is formol saline fixation is all right for a couple of days Weil[106] has shown long ago that phosphate groups are released from the complex lipids when the tissue has been allowed to fix in formol saline for a long time

Lillie[56] has suggested the use of the dye Oil Red O as a fat stain He believes that it is equal in all respects to Sudan Black It is suggested also that Oil Red O is entirely restricted in its staining to fats as such Earlier it was mentioned that there is a possibility that Sudan Black is staining some materials which are not fats

Baker[5] has modified the Smith Dietrich method for phospho lipids[95] and added considerably to its specificity This has been proven repeatedly in sections and can be compared with similar results in the test tube or on fats or other materials which are deposited on a cigarette paper In brief the method of Baker makes use of the ability of phospho lipids to form compounds with hematoxylin

eral reactions as are included in most of the biological staining procedures The second reaction is more specific It depends upon recognition of one or more of the amino acids which compose the protein Under the same heading would be in groups the reactions for the nucleo proteins which depend upon the demonstration of a portion of the non protein portion of the nucleo protein An example of this is Feulgen's reaction which demonstrates desoxyribose nucleo protein by virtue of splitting the protein from the ribose

General reactions for proteins are unsatisfactory This has certainly been true until fairly recently The staining of tissue seen in the ordinary tissue section by almost any dye does appear to be due to amine carboxyl and hydroxyl groups of proteins Eosin in aqueous or alcoholic solution will color a number of tissue constituents which on further analysis can be shown to include the majority of tissue proteins It is doubtful whether this dye or any other one of the dyes or dying mixtures used in classical histology or cytology is staining protein alone

Dempsey Singer and Wislocki [7] have shown that oxidation of a tissue section containing various materials increases the basophilia of the tissue This oxidation is best performed by 1 per cent aqueous solution of periodic acid for one hour at 37° C It is thought that the periodic acid brings about the formation of strongly acid basophilic groups The acid groups themselves have not been precisely identified but they do appear to be formed most readily in reagents of high sulphur content The suggestion of Dempsey and his coworkers is that sulfide and sulfhydryl may be oxidized to the sulfonic acids and hence present an increased basophilia This matter needs considerably more study

A relatively recent development for proteins which I have not had the opportunity to apply personally is Danielli s use of diazonium compounds to demonstrate protein [26] The diazonium compounds form colored products with histidine tryptophane and tyrosine as well as purines and pyrimidines The color is intensified by allowing it to form an azo linkage with some phenol The phenol used by Danielli is b naphthol Danielli has compared the results in tissue sections with test tube experiments Pearse who has used this method believes that it is promising and has illustrated some very fine appearances with its use

The second type of test for proteins makes use of the demonstration of the amino acids constituents of them [95] These include (1) the ninhydrin reaction which takes place with all the amino acids (except proline and hydroxyproline) with peptides proteins and also a large group of other compounds The reaction has the difficulty that the color must be developed by boiling and that the color is diffusable

(2) The alloxan reaction is not sensitive enough An alcoholic 1 per cent solution of alloxan gives a pink coloration with amino acids for protein compounds Again the color diffuses

(3) The tests for sulfhydryl groups may not be specific since SH and S S groups give the reaction

(4) The Biuret reaction is given by peptides when there are at least three amino acids present The strongly alkaline solution damages the tissue

(5) The xanthoproteic reaction is based upon the yellow coloration which is given with proteins by concentrated nitric acid The color of the tissue may be changed to orange by exposing the section to dilute ammonia solution or amonive vapors after the action of the acid This reaction is due to the presence of tyrosine phenylalamine or tryptophane and is also given by all phenolic compounds

used for the demonstration of sterols particularly the ketosteroids As Claesson and Hilart [1] have shown and as Cain has emphasized many of the materials which color with the acetal phosphatide test will color with the phenylhydrazine reagent of Bennet Actually phenylhavdrazine is a non specific reagent for aldehydes The possession of the aldehyde group after mild hydrolysis or oxidation with mercuric chloride and as in the free state has been previously mentioned Some over lapping biologically as well as histochemically appears certain in this type of reaction

Proteins

The proteins are made up repeating structural units These appear to be pep tide chains formed by cross linkages between amino acids Proteins from the one source have a regular repetition of the same amino acid Each amino acid can be considered to be an organic acid in which one or more residual hydrogen atoms have been replaced by amino groups Actually, only forty amino acids have been found in nature although the number of amino substituents of organic acids is almost infinite Some twenty five of the naturally occurring amino acids have been found in proteins The specific proteins of each tissue in each species the numerous viruses and the numberless immune proteins are constructed by the arrangement of these amino acids

Proteins and their breakdown products form colloidal solutions in water The amino acids are water soluble except for leucine tyrosine and cystine Hydroxyl ysine may not be water soluble at all times The colloidal nature of protein and polypeptide solutions indicates that these are large molecules

Simple proteins yield only amino acids on hydrolysis Albumin globulin histones and protamines are examples of this class Histones and protamines are relatively simple proteins being found alone only in lower animals In many they are combined with nucleic acids in the nucleoproteins (p 162) Albumins and globulins are structurally related proteins which differ in their interval struc ture and properties Albumin is water soluble while globulin is not The molecular weight of the albumins and globulins of blood serum have been much studied

Compound or conjugated proteins are units made up of proteins joined to a non protein or prosthetic group Phosphoproteins are compounds of proteins and phosphoric acids Casein in milk is the chief phosphoprotein in the human The nucleoproteins (p 162) are compounds of histone or protamine with nucleic acid Mucoproteins and mucopolysaccharides (p 147) are compounds of protein and carbohydrates Chromoproteins are compounds of protein and a pigment Examples include hemoglobin cytochrome and the flavoproteins

Derived proteins are produced by cleavage and denaturation of proteins In cleavage the protein molecule is dismembered the fragments decreasing in size in the following order metaproteins proteoses peptones and peptides Break down of the peptides yields the constituent amino acids Denaturation is a re arrangement of the protein molecule which alters its solubility The usual histological fixation includes denaturation of the cell protein Denaturation of albumin and globulin can be brought about by heat coagulation in acid medium

The histochemical techniques for proteins fall in general into two groups The first is the demonstration of some particular staining reaction or affinity which proteins appear to have These are relatively nonspecific and include such gen

nuclease according to Brachet [14] It is thought that basophilia which can be re moved by ribonuclease is due to ribonucleic acid As Danielli[3] has pointed out there are three suppositions in this idea (1) that completely pure enzymes can be obtained (2) that the enzyme can attain access to substrate and (3) that the material which is removed by the enzyme is no more than the substrate For the first part the pure enzymes as previously mentioned are difficult to obtain The second problem of the access of the enzyme to the substrate is fairly obvious If there is a substrate covered by a non susceptible material even in a mono layer the substrate will not be attacked by the enzyme The third part seems to me particularly important If a material is present in small quantities but yet of a crucial situation in holding together a cell structure the whole material or whole cell structure might be removed by the enzyme although the substrate itself plays a relatively small part in the structure

Brachet[14] has used the methyl green and pyronin of the Unna Pappenheim stain to differentiate between pentose and desoxipentose nucleic acid The methyl green is supposed to stain with the desoxy form while the pyronin stains with the pentose form The matter has been thoroughly studied lately by Kurnick [51] He presents considerable evidence that under controlled conditions such may actually be the case The recent literature is reviewed by Lumb in the article to which reference has already been made

The identification of the nucleic acids by ultraviolet techniques or absorption techniques has already been mentioned as being outside the scope of the ordinary laboratory of pathology However it should be mentioned at this time that much valuable information is being gained by this method in the research laboratory Caspersson introduced the method and has lately summarized his views in a most interesting monograph Cell Growth and Cell Function [20] Some of the results which are applicable to pathology are included in this monograph It should be required reading for pathologists who are interested in the application of histo chemistry to the problems of their special science At the same time the difficulty of applying these methods to the routine pathological laboratory will appear

Besides these histochemical methods which use sections for study there are a number of micro chemical methods which have been applied to cellular chemistry These include (1) differential centrifugation of tissue homogenates and chemical study of the nuclei mitochondria etc which separate off as layers and (2) histo logical and chemical study of alternate frozen sections of tissue These are de scribed fully in Glick s monograph on Histochemistry They are not suitable for laboratories of routine pathology although valuable for research

ADDENDUM

R D Lillie (Stain Technol 26 123 136 1951) has pointed out that the ace tylation procedure (p 155) may take considerably longer that the forty five minutes proposed by McManus and Cason This discrepancy I cannot explain

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(6) The tyrosine reaction of Millon is given also by tryptophane The color produced by tryptophane is marked by it lasting only a few minutes while that by tyrosine is more permanent

(7) The tryptophane reaction of Voisenet Furth is fairly specific It appears sensitive enough to be of some value

(8) The arginine reaction of Sakaguchi[90] This is the method which has found more use in my own hands and appears most promising for future development The amino acid arginine is widely distributed in nature Tissues which contain large quantities of it include keratinized materials thyroid colloid and collagen The test tube methods of Sakaguchi has been applied by Baker[6] Serra,[9] and by Thomas[101] This original method of Sakaguchi developed a color with b naphthol The more recent publication of Sakaguchi[91] describes the substitution of 8 hydroxyquinoline or oxine in place of the b naphthol This procedure is quite easy and gives repeatable and semi permanent results Warren and McManus[104] applied Sakaguchi s oxine method to histochemistry Thomas in a personal communication has suggested that the method may be modified to increase its sensitivity His publication is awaited

The demonstration of nucleo protein by the Feulgen method is one of the classical procedures of histochemistry[3] It was introduced by Feulgen and Rosenbeck in 1924 It appears specific for desoxyribose or desoxypentose nucleic acids The hydrolyzed sugar combines with Schiff s reagent to produce a purple red or magenta colored compound The mechanics of the method are beginning to be understood

The initial effect of the hydrolysis of desoxypentose nucleic acid by normal hydrochloric acid at 60° C appears to be a liberation of purines The linkage is split between the purine and desoxyribose or desoxypentose sugar The sugar changes from the furanose to the aldehyde configuration The free aldehyde groups are combined with the Schiff s reagent to form the colored compound The optimum time of hydrolysis with normal hydrochloric acid must be worked out for each tissue and for each section The species difference is considerable Generally from eight to twenty minutes hydrolysis in normal hydrochloric acid and at 60° C is sufficient

Stedman and Stedman[98] have criticized the validity of the Feulgen technique for desoxypentose nucleic acid They have suggested that the nucleic acid is located outside the chromatin and that the water soluble dye formed as the result of the Feulgen reaction is physically attached to a specific protein in the chromosone There is very good histochemical evidences such is not the case

Some of the materials in sections may give a color with the Schiff s reagent without previous hydrolysis Weak hydrolysis with mercuric chloride as mentioned previously may also release materials which combine with the Schiff s reagent to produce a color For these reasons control sections should be carried through Lumb[64] has reviewed the evidence for and against the specificity of the Feulgen reaction and it is highly probable in her eyes as it is in the opinion of most investigators that the Feulgen reaction is specific with desoxypentose nucleic acid She does point out however that the precise localization of the nucleic acids should be accepted with some reservations and if possible checked by comparison with other cytochemical tests

The demonstration of ribose or pentose nucleic acid has depended in large part upon the basophilia of this material The basophilia can be removed by ribo

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PART II—ENZYMATIC HISTOCHEMISTRY

Cellular Metabolism

(1) **General Considerations**.—The cell is described chemically as composed of organized aggregations of fat, protein and carbohydrate. The dissimilarity of component parts is present at a microscopic level—the mitochondria, for example—and at a molecular level—the nucleoprotein. Structural complication is matched by metabolic complexity. The cellular chemicals are in a dynamic state, constantly renewing and replacing parts in a remarkably efficient manner. The integral action of dissimilar parts and the efficiency of cellular processes depend alike on enzyme process.

It is a sort of balance which is being sought in cellular activity. The opposing force of synthesis and analysis are continually at work, yet the attainment of equilibrium is never quite complete. The energy necessary for life is furnished by this nicely balanced struggle between breakdown—catabolism—and build up—anabolism. Life itself may consist in no more than the persistence of reversible reactions. The end product turns the direction of the process to the formation of the parts. These in turn combine in the end product, and so on.

Protein and nucleoprotein especially appear to play the major role in cellular metabolism. True or not, as time will tell, it has been and is the fashion to consider the functioning of cytoplasm as a matter of synthesis and analysis of protein. Nucleoprotein makes up nucleus and nucleolus as well as forming the central structure of the mitochondria and chromidia or microsomes. From a philogenetic point of view, the simplest forms of life—the viruses—contain nucleoprotein. Many viruses appear to be entirely made up of it.

It is impossible for the carbohydrates and lipids of the cell to have passive and storage roles only. It can be shown that these materials take part in metabolic changes and in disease processes. However, considering the cell teleologically, it is the nucleoproteins which are the most characteristic, as well as essential parts. Most of the cellular activities can be oriented to the renewal and breakdown of the nucleoproteins, to their alteration in folding and unfolding or to their increase in size and number, etc.

(2) **Enzymes and Enzyme Activities**.—Enzymes in general are thermolabile organic protein compounds elaborated by living beings. They are capable of initiating and/or accelerating a chemical reaction without themselves being destroyed. Their influence on the reaction is that of catalysis. Most simply stated, enzymes are organic catalysts.

Enzymes occur in all living cells. Special cells with high concentrations of certain enzymes are found in those organs with digestive function. Actually an enzyme must exist for every organic compound found in the cell. Genetic constitution appears to control the presence of enzymes in the mold, neurospora.

A high molecular weight is characteristic of enzymes. All are of colloidal nature. They have isoelectric points and behave amphoterically, both features depending on their protein nature. Enzyme reactions follow chemical laws and can be varied by temperature, concentration, etc. The material acted upon is called the substrate. There is a marked specificity of enzyme for substrate, this specificity being especially marked in the intracellular synthetic enzymes. Other

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The constant struggle for equilibrium produced by anabolism and catabolism make the condition of the cell under resting conditions ' Dynamic Equilibrium '

The Phosphatases

These enzymes will be first discussed because of their very considerable ease of demonstration, their importance in the metabolic cycle and the very many studies which have been devoted to them It is proposed to describe the general principles of demonstrating these enzymes to attempt to assay their role in cellular metabolism and finally to give some of the results of their study in tissues

In general the phosphatases are demonstrated by incubating a section of tissue in which the enzyme is preserved in a solution containing the substrate an appropriate buffer system and a material which will precipitate the phosphate split off by the phosphatase Activator materials may be included In the classical method of Gomori for demonstrating alkaline phosphatase, the glycerophosphate is buffered with sodium barbital and magnesium is present as an activator, while calcium chloride is present to precipitate the phosphate split off as the insoluble calcium phosphate The subsequent demonstration of the calcium phosphate deposited as such at the sites of phosphatase activity may take several forms Gomori originally used the dye Acridine Red to color cobalt which had replaced the calcium deposit This procedure included a histological differential washing which decreased the histochemical validity of the method The same worker later introduced the demonstration of the sites of enzyme activity as the black cobalt sulfide by an ammonium sulfide wash This procedure is open to question since Doyle and Holter *et al* have shown that not all the cobalt is changed to the insoluble black sulfide some being oxidized to the sulfate Kabat and Furth use van Kossa s method for calcium to demonstrate the calcium phosphate using a silver salt which is reduced by light Menten has used an azo dye which is precipitated as the colored compound for example using calcium β napthol phosphate

These various methods all give about the same results with the one species and organ if the earlier handling of the tissue has been about the same It has been the general custom to use paraffin sections of alcohol or acetone fixed tissues or frozen dried material Throughout the procedure it is important that the tissues do not encounter any poison such as formalin or mercury or be exposed to a temperature above 58° C since inactivation will occur It can be shown that there is a loss of enzyme activity at every point along the progress of the tissues in paraffin embedding through alcohol and benzene as many authors have pointed out

I have lately turned to the use of frozen sections of unfixed tissue or tissue fixed briefly in alcohol and then washed However this method has the disadvantage that all tissues are not well handled and paraffin sections can be used provided it is realized that about as many sites of blackening with the cobalt method appear after overnight incubation in a paraffin section as appears in one hour in the same tissue in frozen section

The phosphatase with an optimum pH of activity on the acid side is shown in essentially the same fashion with the calcium being replaced by lead This is the so called acid phosphatase It is noteworthy that fresh prostate contains so much of this 'acid phosphatase that some activity can still be demonstrated at an alkaline pH

enzymes attack *groups of materials—the proteins by proteinases* the lipids by lipases etc In general the enzyme is termed by adding the suffix ase to the name of the substrate, *e g* peptidase attacks peptides esterase attacks esters, etc

There is some evidence *that the chemical configuration of the enzyme resembles* that of the substrate While all enzymes are presumably protein, enzymes acting upon carbohydrates may have a carbohydrate moiety For example hyaluronidase may contain *a carbohydrate closely resembling the substrate hyaluronic* acid

Enzymes occur in some cells in an inactive or zymogen form An activator—the kinase—is needed by *some in others metals such as Co or Mg are required* Some enzyme systems have their activity increased by "protector substances These are presumed to remove or inactivate inhibiting substances

Enzymes are inactivated by many means *Heavy metals—Ag Hg and Cu*—and some ions—H^+ or CN^-—in other instances can poison enzyme systems In the case of the heavy metals it is presumed that binding of the sulphydryl group in the enzymes occurs Irradiation inactivates some enzymes Heat kills most enzymes

There is inhibition of enzyme activity by the law of mass action *i e* over production of substrate On the other hand some inhibitors are known which can combine with the enzyme in place of the substrate *i e* competition with the substrate for the enzyme Inhibiting factors for enzymes occur in nature *e g*, the ascaris has materials inhibiting digestive enzymes

(3) **Energy Production in the Cell** —The energy metabolism of the cell takes place at a sub microscopic level Energy for maintenance of life as well as that used in the elaboration of special products is derived from the constant enzymatic activities of the cell The microscopic and submicroscopic organization of the cell is important in many of these enzymes some not functioning in cell free filtrates Surface relationships and spatial arrangements may play a part as well in enzyme rate

Fats carbohydrates and proteins are utilized in the cellular production of energy Oxygen can be used but not by all cells There is some evidence that carbon dioxide is incorporated in living cells under some conditions The route of utilization of the energy producing materials is a complicated one Not all the details are known There is some evidence not yet complete that energy utilization is similar in *all living cells Description of the* whole process would be out of place in this chapter Details may be found in the references at the end of this chapter A few salient features will be presented

In *cellular metabolism there are common pathways by which carbohydrate* fat and the amino acids furnish energy Phosphate bond energy is utilized by electron transfer Phosphate energy is stored as adenosine adenylic acid the di and triphosphate In the breakdown of fat and carbohydrate pyruvate and lactate are important intermediates Acetate may be an end product of both carbohydrate and fat breakdown possibly also of protein analysis

Accelerating the cellular energy processes are such materials as the co-enzymes—nucleic acid derivatives—and the cytochrome system All the reactions concerned are reversible and synthesis can take place by reversal of the breakdown route Protein synthe is requires energy from fat and carbohydrate oxidation The other syntheses appear self supporting Elementary fats carbohydrates and amino acids appear to be usable by the cell as sources of food and energy

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solution activated by $CaCl_2$, $MgCl_2$ and $MnCl_2$. The second method for the cholinesterase of tissue is that of Friedenwald and Koelle. These authors use fresh frozen sections (while Gomori used paraffin sections of acetone fixed tissues) and use acetylthiocholine as a substrate in the presence of copper glycinate at pH 8. The copper thiocholine produced by the action of the enzyme at the sites of enzyme activity is colored to brown copper sulfide.

There are certain difficulties in the acceptance of these histochemical techniques for cholinesterase. One which is mentioned by Dounce is the failure of the enzyme from the electric eel to split the substrate used in Gomori's method. However the cholinesterase in tissue sections can be inhibited by the various inhibitors such as prostigmine and poisons such as the nerve gas poisons. This latter effect can be shown in vivo and on sections.

Hard and co-workers and Koelle and Friedenwald have used the selective inhibitors of AChE and ChE to study the localization of these enzymes in tissue sections. It can be shown that cholinesterase is present in the ganglia and at the nerve endings and in the nerve tissue especially the gray matter. In the method of Friedenwald and Koelle the danger of diffusion is obviated by the dropping of the frozen section into the substrate mixture which has been saturated with the reaction product, in this case copper thiocholine, before the section has a chance to thaw.

These methods for cholinesterase have a very considerable promise for the pathologist in the study of the neuro-muscular diseases such as myasthenia gravis and the muscular dystrophies as well as many nervous diseases. It may be that these methods will be useful in explaining the pharmacological action of many materials.

B-Glucuronidase

Conjugation with glucuronic acid is one of the ways in which the body rids itself of foreign and toxic materials. Glucuronic acid polymers are important constituents of body tissues making up the ground substance in part and the other body carbohydrates such as the basement membranes and reticulin in all probability. The enzyme which controls the metabolism of the B-glucuronic acid compounds, their breakdown and perhaps their synthesis, is called B-glucuronidase.

The enzyme is widely distributed in nature and in the human body, greatest concentrations being found in the liver and spleen. It is present in the blood serum. The endocrine glands contain a large amount. In the endometrium there is a wax and wane with the menstrual cycle and after the menopause the uterus and vagina show very low values.

Histochemical methods for the demonstration of B-glucuronidase were developed by Friedenwald and Becker. They took advantage of the formation of glucuronic acid complexes with foreign organic materials which the rabbit is fed and which he excretes as glucuronides in the urine. This is known as the biological method of preparation. The first substrate used was ortho amino phenyl glucuronide which was split with the formation of an insoluble red precipitate at the sites of enzyme activity. The second and preferable substrate was 8-hydroxyquinoline glucuronide which was used with ferric iron. The ferric iron precipitated the 8-hydroxyquinoline as a ferric complex which was then demonstrated

substrate solution Tissues in formalin up to three months can be used This reversal of the inactivating effect of formalin is in keeping with our ideas about the reversible nature of the binding of protein with formalin It has not been extended to other enzymes to my knowledge

Gomori has shown that lipase can be demonstrated in the normal lung and liver, in the intestine and in the pancreas In the latter situation there is a localization in the zymogen granules The salivary glands and stomach were other situations in which lipase was found From the viewpoint of pathology, relatively little use of this valuable method has been made Gomori localized the lipase in the tubercle to the giant cells and epithelioid cells, which McManus pointed out were the sites at which granules of lipoid demonstrable with Sudan Black could be found A similar association of lipase and lipid is seen in the atherosclerotic plaque where the lipase is demonstrable in the inner portion of the plaque Mark has studied the lipase distribution in experimental rat hepatic tumors Menke has done the same for some human pulmonary cancers

The method for lipase has been unsatisfactory in the hands of many workers Others have found the method useful, suggesting that there is still some art to the science of histochemistry

Choline Esterase

Recent studies have emphasized the importance of choline esterase in the transmission and mediation of the nerve impulse while the wide occurrence of acetyl choline in tissues has awakened the search for enzymes taking part in its metabolism Choline esterase is found especially at the "end plate" of nerve termination on striated muscle and in the electric organ of the electric eel In the nerve fibers there is some evidence that the splitting acetyl choline initiates the electric charge which constitutes the nerve impulse

Dale in 1914 hypothesized the existence of an enzyme capable of splitting acetyl choline The very considerable studies which have elucidated the nature and significance of such enzymes are too numerous to detail They have been well summarized by Augustinsson from whose review much of the following material is derived

Modern terminology of this group of enzymes has spoken of "true" and "false" (or pseudo) cholinesterases As Augustinsson points out there is some overlapping of activity in regard to a number of substrates and it is better to speak of the enzymes which can break down acetyl choline in some other terms The same investigator with Nachmansohn suggests the term Acetyl choline esterase (AChE) to describe the enzyme which occurs in nervous and muscular tissue and in erythrocytes This is the "true" cholinesterase of other workers The other esterases are termed Cholinesterase (ChE) as a group title These are found in some sera in pancreas and salivary glands etc A point of some importance to the histochemistry of the Cholinesterases is that materials are known which selectively inhibit the AChE or the ChE in the tissues

Gomori developed the first method by which the tissue cholinesterase could be shown The principle is that of the phosphatase methods the tissue sections split a substrate which releases a precipitable material which is in turn demonstrated The first substrate of Gomori was a choline ester of a fatty acid such as myristoyl choline It was precipitated by cobaltous acetate which was present in a buffered

sweat glands, leucocyte granules and ganglion cells The substrate for the dopa oxidase has not been identified in blood or in many other situations where it might be expected It may be that the tyrosine is brought by the blood to the tissues and dopa is produced in the skin and in these other situations where the enzyme is found

The enzyme is demonstrated histochemically by observing the formation of the black pigment upon incubating fresh unfixed tissue with the substrate that is di hydroxyphenylanine for a period of one to six hours There is quite a wide range of pH activity The slowest formation is at pH 7 4 Phosphate buffer can be used Actually as Mallory s text book points out the action can be carried on from 7 4 to 8 2 At 8 2 the reaction occurs in only one hour at body temperature while at pH 7 4 the action is completed in four to five hours Mallory however, enters a step which would decrease the histochemical specificity of the reaction It is suggested that the reaction mixture in the incubator at 37 5° C should be inspected every thirty minutes or so In two to three hours the solution is reddish and in three to four hours a deeper brown At the end of the development of the brown color, the reaction is said to be complete This would put a differential feature in a histochemical procedure and would decrease the validity of the method The change in the color of the incubating solution will occur spontaneously if the solution is kept in daylight The accelerated change in the color of the solution is presumably due to the spontaneous change as well as to some diffusion of enzymes The appearance of dopa oxidase in anomalous situations such as white cells and muscle is said to be due to polyphenol oxidase

The dopa oxidase enzyme as demonstrated histochemically occurs both in cytoplasm and in nucleus This latter situation is difficult to understand unless it is the result of the diffusion which was mentioned previously

Peroxidase

The peroxidase reaction in human tissues is actually the cytochrome oxidase The method which first showed this enzyme was that of Ehrlich Ehrlich s old 'Nadi reaction made use of alpha naphthol and dimethyl p phenylenediamine By this method the sites of oxidase activity stain a deep blue It was shown by Hollande that the dye which was produced by the oxidase reaction is capable of fat staining Indophenol blue as it is called is quite a good fat stain There was a considerable argument concerning the staining of the granules as whether due to simple fat staining or due perhaps to the enzyme reaction At the present time it appears most reasonable that the stained granules particularly in the white blood cells are colored as the result of enzmye activity

Ritter and Oleson have applied the method of the peroxidase reaction to paraffin sections They have used the block procedure of staining before embedding They use alpha naphthol and Superoxol to demonstrate the peroxidase granules

It is still possible sometimes to demonstrate the peroxidase as cytochrome c oxidase granules and cells after fixation in such materials as formalin vapors but as a rule alcohol and certainly xylol destroys the enzyme Therefore if the peroxidase is to be found in tissue sections the tissue section must be prepared from unfixed tissue by frozen section technique or by smears or the peroxidase reaction must be done first according to the method of Ritter and Oleson

by the familiar prussian blue reaction Both substrates are used in acetate buffer at pH 5 0

The enzyme appears in the cytoplasm rather than nucleus It is seen in the kidney tubules liver parenchymatous cells, nodules and pulp of spleen, in the bronchial mucus membrane and alveolar walls in endometrial glands and in cartilage and bone It can be seen that the distribution of this enzyme is very wide and that it occurs in many situations in which complex carbohydrates are found The demonstration of increased activity in tumors which was shown by Fishman in the test tube can be confirmed in sections Campbell did this and showed that experimental carcinomas were high in B glucuronidase activity Campbell showed that the enzyme was concentrated at the periphery of the tumor This suggested that the enzyme might be concerned with invasion rather than with the metabolism of steroids, as Fishman suggested In the studies of Campbell, the second substrate of Friedenwald and Becker was used

B glucuronidase needs frozen sections of unfixed tissue for its demonstration The pathologist uses this type of section very frequently and studies many tissues upon which the method of Friedenwald and Becker would be used with definite information produced

"Dopa-oxidase"

Dopa oxidase demonstration in tissues is one of the older and more standard methods of tissue pathology It is very useful in indicating the non pigmented types of melanomas A few chemical considerations are necessary to the understanding of its use

Melanin is a chemical term which covers a wide variety of pigments in the animal kingdom A discussion of the cutaneous melanomas that is the malignant tumors arising from the melanin producing or containing cells is found in another chapter of this book (p 28) The word melanin is derived from the Greek *melanos* meaning black but chemically it is taken to mean any pigment In the biological families the black insoluble pigment which we call melanin is widely distributed in nature It is found in the retinae of mammals the hair horn and feathers the skin and in the central nervous system In the man only is the pigment of the substantia nigra a melanin The melanins are also found in the ink of the octopus The discolorations of apples and potatoes are closely related or identical pigments Their chemical nature is uncertain They are probably a group of material related to tyrosine These actually make up polymerized indoles

It is interesting that one of the melanins hallochrome has been identified only in red hair red poultry feathers and the pigment of certain annelid worms

The indole ring is a pyrrole joined to a benzene ring This indole structure is found in melanin The amino acid tyrosine is a closely related compound There is some discussion as to whether the dopa oxidase enzyme of the pathologist is not actually a tyrosinase The dopa oxidase is not specific for dopa or dihydroxyphenylanine but will break down to a black pigment tyrosine and closely related compounds The enzyme tyrosine will in turn break down dopa

Mammalian skin contains no tyrosinase A dopa oxidase can be demonstrated in many of the cells Dopa oxidase is found also in a number of tissues where its occurrence is difficult to understand Examples of these situations are muscle

Chapter

7

CORONARY ARTERY DISEASE

By J. C. Paterson, M.D.

Coronary artery disease is not a pathological entity; it is a clinical term for a number of symptom complexes which result from myocardial ischemia. And the ischemia, in turn, arises from a disproportion between the nutritional requirements of the heart on the one hand and the amount or quality of blood which is delivered through the coronary arteries on the other. Depending upon the degree and duration of this disproportion various syndromes may be produced.

The *anginal syndrome* occurs when the disproportion between supply and demand is of moderate degree and of short duration. It is characterized by transient chest pain which is usually precipitated by effort and relieved by rest. The *syndrome of coronary occlusion* occurs when the disproportion is of longer duration. From the experimental evidence the ischemia must last for at least twenty minutes. This syndrome is characterized by prolonged chest pain of crushing intensity accompanied by signs and symptoms of circulatory collapse. The *syndrome of acute coronary insufficiency* is found in those individuals who die suddenly from "heart attacks" without premonitory symptoms. Its mechanism is imperfectly understood but here the disproportion would appear to be acute and extreme and there is reason to believe that it is accompanied by myocardial irritability which results in ventricular fibrillation.

Many factors may be concerned in the disproportion between supply and demand and either one or several may operate in an individual case. Factors which decrease the nutritional supply to the heart include narrowing or occlusion of the coronary arteries, a sudden fall in blood pressure from shock, and a lowered oxygen capacity of the blood from anemia. Still other factors increase the work of the heart and call for greater nutritional requirements. The most important of these are effort or excitement, arterial hypertension with cardiac hypertrophy, and valvular deformities.

These are the factors which are concerned in the production of the syndromes of coronary artery disease, but one of them far outnumbers the others in frequency — a mechanical obstruction to the free flow of blood through the coronary circulation. Here again there are rare and common causes. The rare obstructing lesions are coronary embolism, syphilitic aortitis with stenosis of the coronary ostia, non-specific or rheumatic arteritis, periarteritis nodosa, and thromboangiitis obliterans. The only common cause is narrowing or occlusion of the coronary arteries from arteriosclerosis and its sequelae. There is reason to believe that this disease process is at fault, wholly or in part, in over 90 per cent of the cases of coronary artery disease. Indeed, some authors use the terms coronary artery disease and coronary arteriosclerosis synonymously. The present chapter

Keilin was the first to show that the 'Nadi' oxidase or the peroxidase enzyme was identical with the cytochrome oxidase system It can be shown, for example, as Moogk has done that azide will specifically poison the peroxidase or cytochrome c oxidase enzyme in tissue The diffusion of the stain is considerable as Glick points out and the possibility of false reactions are always present so that control preparations poisoned with azide should always be used

The chemistry of cytochrome is a complicated one The identity of cytochrome oxidase with the yellow ferment of Warburg has been suggested The present opinion is that the cytochrome is reduced to free some oxygen which in turn oxidizes the reagents of the peroxidase or cytochrome c oxidase system In a few preliminary studies of my own it has not been possible to enhance the reaction by the addition of cytochrome or oxidized cytochrome to the mixture of substrate and frozen section of tissue There are a number of anomalous results such as the absence of the histochemical demonstration of the enzyme from heart muscle In heart muscle it would be expected to be in very high concentration It has not been possible to demonstrate this It may be that the enzymes concerned are destroyed quickly after death and that surgical specimens or very fresh autopsy material should be used I have not attempted this histochemical procedure on material which has been fresher than several hours after death

A number of significant histochemical articles and reviews have appeared since the completion of this section These will be found scattered in many journals A noteworthy review is Volume I International Review of Cytology, ed G Bourne Academic Press, 1952 in which appear articles by Montagna on the histochemistry of the skin by Gomori on the esterases, etc Several of Seligman s articles on the histochemistry of enzymes appear in Cancer 1951 and 1952 and along with other subjects in the proceedings of the meetings of the Histochemical Society published in Journal of National Cancer Institute 1950 1951 and 1952 Laboratory Investigation editor T J Kinney Hoeber N Y C publishes many histochemical articles and the Journal of Histochemistry and Cytochemistry editor R D Lillie Williams and Wilkins Baltimore begins publication January 1953

REFERENCES—PART II—ENZYMES

The references on enzymological studies summarized here would be too voluminous if given *in extenso* The following general references plus those on enzymes given in the first portion should serve as an introduction to enzymatic histochemistry

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evidence of disease. However, they are of questionable functional significance since when normal coronary arteries of man are plugged by thrombi or emboli or when those of animals are artificially occluded a myocardial infarct (or sudden death) almost invariably results. The rapid necrosis of cardiac musculature in these circumstances could hardly occur were adequate anastomoses present.

Quite a different type of anastomotic circulation develops in the presence of coronary arteriosclerosis. On injecting a lead agar mass, which is so viscous that it penetrates only those channels which are 40 microns or more in diameter, Schlesinger and his group have clearly demonstrated that a functioning circulation develops when and where it is needed. If the arteries are gradually narrowed by disease, anastomotic channels of some magnitude are formed about the points of stenosis and there only. These larger channels are never found in normal hearts nor in those parts of diseased hearts in which the circulation is normal. Since the arteriosclerotic process is characteristically irregular in its distribution and affects the major coronary branches to different degrees, the complexity and variability of the anastomotic channels appear to be almost unlimited. In fact there is reason to believe that they may compensate for narrowing or occlusion to a degree sufficient to enable the heart to meet the demands of the ordinary activities of life.

The development of a collateral circulation within the heart would appear to be slow, and it should be maximal in those cases in which the coronary lumina are gradually encroached upon by slowly enlarging arteriosclerotic plaques. Whether or not myocardial infarction will occur as a result of sudden occlusion of an artery by an embolus, a hematoma, or a thrombus will depend upon the extent to which an anastomotic circulation had developed prior to occlusion. In general, it seems evident that when occlusion progresses more rapidly than the collateral circulation develops, myocardial anoxemia and necrosis will be the rule.

These findings have important clinical applications. Uncomplicated and progressive coronary arteriosclerosis leading to marked stenosis of major coronary branches will not necessarily result in symptoms since it is compensated for to a degree by the opening up of an efficient anastomotic circulation. Conversely, an attack of coronary occlusion, if not immediately lethal, will be followed by the development of a collateral circulation, by an increase in the "coronary reserve" and by the disappearance of the symptoms of functional insufficiency. The prolonged convalescence that is usually prescribed for a case of coronary occlusion will thus serve a double purpose. Not only will it minimize the chance of cardiac rupture at a point of infarction, but it will also allow time for the development of an efficient collateral circulation.

The natural trend of a case of coronary artery disease is therefore towards improvement or recovery, and this must be kept in mind when the efficacy of suggested therapeutic procedures is being tested. Not only must such tests be rigidly controlled but the experimental series must be large enough to eliminate the effect of individual variation. The factor of individual variation is particularly important as Schlesinger and his group have shown that the complexity and variability of distribution of the component vessels of an anastomotic circulation are almost unlimited. One suspects that many of the "cures" of coronary artery disease which have been claimed from vitamin E therapy have resulted more from the the natural development of a collateral circulation in the heart than from any inherent efficacy in the therapeutic agent. That, at any rate, is my

then will be limited to a discussion of the etiology, the pathogenesis, and the sequelae of arteriosclerosis in general and of coronary arteriosclerosis in particular

Coronary arteriosclerosis is almost always of the atherosclerotic variety, and as such it does not differ essentially from the same process in other arteries However, it has some special features which give to it a peculiar importance The atherosclerotic process in general is characterized by rounded intimal plaques which project into the arterial lumina and retard, to a degree the tree flow of blood And because the coronary arteries are relatively small vessels, plaques of any given size will produce more embarrassment than they would in larger vessels like the aorta Of even greater importance is the unusually vital nature of the tissues supplied by the coronary arteries When they are narrowed or occluded by the arteriosclerotic process serious clinical manifestations may ensue—chronic invalidism from angina pectoris, prolonged incapacitation from coronary occlusion, and worst of all, sudden death from acute coronary insufficiency The high incidence of these clinical effects in the population of temperate zones an incidence which appears to be increasing in recent years has called forth a mounting interest in the study of the underlying disease process And this interest has been stimulated and accelerated by the formation of learned societies in which the rapid exchange of information between investigators is achieved, and by the provision *of ample funds for research by government agencies and charitable organizations*

As a result of this interest research on arteriosclerosis and particularly on coronary arteriosclerosis has developed rapidly, and it has not been entirely unproductive The present chapter will record the advances that have been made in this field during the past twenty years Points of special interest particularly those of an experimental, pathological and clinical nature will be emphasized namely additions to knowledge in the anatomy and histology of the coronary circulation in health and disease the factors which may be concerned in the acceleration of the arteriosclerotic process (with the hope of course that the process may be reversed) and the manner in which coronary occlusion is produced Two other subjects of a more controversial nature will be discussed briefly the so called premonitory symptoms of coronary thrombosis and the medico legal aspects of some of the effects of stress upon the heart

MORPHOLOGICAL ASPECTS OF CORONARY ARTERIOSCLEROSIS

Even in the field of pure anatomy certain fallacies have been exposed Since the days of Cohnheim the coronary arteries have been popularly regarded as end arteries and it was not until the present century that an anastomotic circulation within the heart was demonstrated[65] The functional significance of this collateral circulation the circumstances which lead to its development and its exact anatomical architecture have been studied in detail by Schlesinger and his co workers[4 53] using special injection methods

If colored solutions of a watery consistency are injected into one major coronary artery of a normal heart some of the material can be clearly visualized in the branches of the other major artery Thus it seems evident that fine communications regularly exist between the major coronary arteries of hearts which show no

cept It should be noted though that intimal vascularization does occur in the later stages of arteriosclerosis, as will be described in detail in this section

Along the same line is the suggestion that the earliest lesion is a tiny mural thrombus which is laid down upon the surface of the intima it becomes covered by endothelium, its contained red cells disintegrate and the initial lipid deposit is thus explained [17] But here again mural thrombi have never been observed in normal arteries although they do form as a result of arteriosclerosis

The results of animal experimentation have not clarified the position regarding the earliest lesion in man The feeding of cholesterol to rabbits leads eventually to the formation of lipid cushions in the intima but these have been shown by Duff to be antedated by degenerative changes in the underlying medial coat [14] The position has been further complicated by the findings in chickens Although it has been claimed by Dauber and Katz that arteriosclerosis in this species may be initiated by cholesterol feeding [10, 11] there is reason to believe that the earliest lesion is due to quite a different cause The coronary arteries of chickens are highly susceptible to spontaneous arteriosclerosis Fibrous intimal plaques without contained lipid can be demonstrated in over 70 per cent of young birds which have been reared on normal diets and it has been shown by serial section technique that foci of medial disease precede the development of the intimal thickenings [47] The primary medial lesions in turn appear to represent points of lymphomatous infiltration of the vessel wall [48] Lymphomatosis is generally regarded as a naturally occurring neoplastic disease of birds the causative agent is filtrable and it can be transmitted from bird to bird by direct contact and from parent to offspring by way of the egg The disease may manifest itself in several ways neural visceral ocular and osteopetrotic forms have been described, and there is reason to believe that a vascular type also exists and that it is extremely common Thus spontaneous arteriosclerosis of chickens can be interpreted as a secondary intimal proliferation in response to the presence of lymphomatous cells in the wall of the vessel These findings in chickens are reminiscent of Virchow s concept of the genesis of human arteriosclerosis that the thickening of the intima of arteries is a reactive proliferation of connective tissue cells in response to an underlying inflammatory process However, it is important to realize that lymphomatosis is confined to the avian family it has never been transmitted to mammals and there is therefore no reason to believe that its causative agent is concerned in the etiology of human arteriosclerosis However, once the arterial *disease has been initiated the mechanism of development of lesions in the two* species may still be similar

Once the human arteriosclerotic process has started there is general agreement upon its morphology The intima is thickened by connective tissue and in it there are usually many lipid containing phagocytes The fibers of the internal elastic lamina are split broken or completely destroyed As the plaque enlarges its central portions show evidence of degeneration until eventually a core of soft atheromatous material rich in cholesterol is formed The plaque has now reached an appreciable size and the pressure of blood upon it, forcing it intermittently against the resistant adventitia results in a thinning out of the media apparently by pressure atrophy

While these changes are going on important alterations in the blood supply to the arterial wall occur The wall of a normal human artery obtains its nourishment from two distinct sources The adventitia and outer third of the media are

opinion and it is supported by the results of controlled experiments by several clinical groups.

Another purely anatomical feature of the coronary circulation—the points which are susceptible to injury by trauma—deserves some discussion. Theoretically, direct violence to the chest with injury to a major coronary artery lying in the line of force of the blow may lacerate the arterial wall and lead to thrombus precipitation and to the syndrome of coronary occlusion. From the medicolegal point of view, it is important to appreciate that the vessel most liable to direct violence of this type is the proximal portion of the right coronary artery which runs across the anterior surface of the heart, just behind the sternum at the level of the second and third interspace. On the other hand, the proximal portion of the left anterior descending branch, which is the most common site for naturally occurring thrombosis lies farther back and to the left, and is protected from direct violence by the root of the pulmonary artery. The terminal portion of this branch does lie anteriorly but it is doubtful if serious consequences would ensue from its injury. Likewise, the left circumflex branch another common site for naturally occurring thrombosis lies to the side of the heart and should thus be immune to the effects of trauma. In a fatal case of unequivocal traumatic coronary thrombosis, then autopsy should reveal a thrombus which will probably *lie in the proximal part of the right coronary artery*. *In addition* the age of the oldest part of the thrombus should agree with the interval between trauma and death, the site of primary thrombus precipitation should be demonstrated at a point of laceration of the arterial wall and there should be evidence of contusion of the adjacent epicardial tissues. Failure to demonstrate all of these points and they call for painstaking pathological examination should lead to the suspicion that the occluding thrombus developed from natural causes. If an individual survives a bout of cardiac infarction suspected of being due to traumatic coronary thrombosis only opinions can be voiced. Even if there is a direct continuity of symptoms following trauma to the chest together with electrocardiographic evidence of infarction in the distribution of the right coronary artery the possibility that the occlusion developed from natural causes cannot be excluded.

The relative strength of the various coats of a coronary artery has recently received some attention. It would appear that the tensile strength of musculoelastic arteries lies principally in the adventitia not in the media. Incision of this outer layer allows the artery to balloon and rupture even at low pressures.[6] Providing then that the adventitia of a coronary artery is not weakened by disease any space occupying lesion within its wall will be projected inward and will tend to stenose the lumen. This stenosing effect will apply not only to the gradually enlarging plaques of arteriosclerosis but more particularly to sudden hemorrhages into these plaques which will augment appreciably their bulk.

The intimate microscopic picture of the arteriosclerotic process in the coronary arteries will now be reviewed in some detail. The earliest lesion in these vessels and in others that are susceptible to atherosclerosis is still obscure. Several suggestions have been made but these are hardly acceptable. It has been claimed for example that the rupture of nutrient capillaries within the intima results in a hemorrhage which on disintegration accounts for the initial deposit of lipids in this layer.[8] However the intima of normal human arteries has no capillary blood supply, and there is therefore no morphological or theoretical basis for this con

grow into the plaques and ramify within their substance Their direct origin from the main arterial lumen was noted first in the aorta [5] they have been shown by serial section technique to arise in a similar fashion in arteriosclerotic coronary arteries [23,4] and the full extent of their profuse ramifications in intimal plaques has been demonstrated by special injection methods [6] The microscopic appearance of intimal vascularization is illustrated in Figures 36 and 37

The direct origin of intimal capillaries from the lumen of major arteries is of particular importance It means in my opinion that the pressure of blood in the main arteries will be reflected to a degree into thin walled channels which are not equipped to withstand pressures of this magnitude The tendency of intimal capillaries to rupture in certain circumstances and to produce intramural hemorrhages and other sequelae, will be discussed at length in later sections of this chapter

FACTORS CONCERNED IN THE ACCELERATION OF THE ARTERIOSCLEROTIC PROCESS

Two points of major importance will be discussed in this section first the reasons as we know them for the initiation of arteriosclerosis and second the factors which are concerned in the progression of the disease once it is initiated

The factor or factors responsible for the initiation of the arteriosclerotic process in man is still obscure Little is to be gained from a detailed examination of the various theories that have been advanced over the past fifty years and all that will be attempted is a brief refutation of some of them

The *senescence* theory that the disease is merely the result of physiological aging processes has now been abandoned While strongly supported by the older German pathologists the not infrequent demonstration of severe grades of disease in children and young adults particularly in the coronary arteries and the finding of only minimal changes in some elderly persons make this theory no longer acceptable

The *hypercholesterolemic* theory of Anitschkow that plasma cholesterol in excess infiltrates the arterial wall at points of mechanical stress and thus initiates the disease is now viewed with skepticism Recent investigations to be discussed later in this section have shown that the critical factor for the development of arteriosclerosis in some animal species is not the blood cholesterol level but is rather the instability of serum cholesterol or some abnormality in its physico chemical makeup

The *lipophage* theory of Leary has few advocates today By this doctrine the initial lesion is caused by cholesterol containing lipophages elaborated in the reticuloendothelial tissues of the liver spleen adrenal glands and lung which are specifically attracted to the arterial wall and penetrate the intact endothelium The validity of this theory has been attacked in various ways notably by the fact that the lipophage accumulations in the viscera and blood in Niemann Pick s disease and in Gaucher s disease do not give rise to arteriosclerotic plaques *

Since this chapter was written the lipophage theory of Leary has been further discredited by the observations of Payne and Duff (Arch Path *51* 379 1951) Rabbits which were fed cholesterol and given intravenous injections of a detergent Tween 80 developed tremendous foam cell accumulations in the reticuloendothelial system However these foam cells did not appear to localize in the walls of arteries as the aorta showed only minimal deposits of lipid

supplied with blood through the vasa vasorum which penetrate the muscular coat from without The remainder of the arterial wall its inner portion is nourished by the imbibition of nutrient materials from the blood circulating in the lumen of the artery The intima of a normal human artery has therefore no true blood supply, although it develops one as a result of disease Apparently because of a compensatory mechanism, to supply the extra nutritional requirements of an abnormally thickened intima in the later stages of arteriosclerosis, capillaries

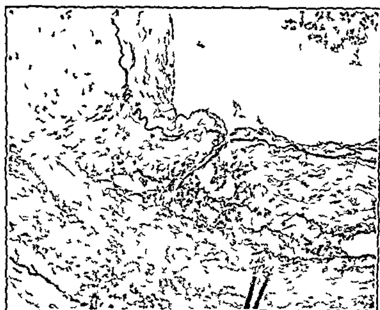


FIG 36 —*Direct origin of a capillary from the lumen of a major coronary artery*



FIG 37 —*Vascularization of an arteriosclerotic plaque* Dilated and congested capillaries ramify in the inner portion of an atheromatous focus

then is the problem of practical importance why do the lesions in some individuals progress to the point that they produce symptoms, while in others they remain small and innocuous? No fixed rules can be offered but from the evidence at hand it would appear that the following factors play some part in the progression of the arteriosclerotic process—the general state of bodily nutrition, the blood lipid level the ratio of blood lipids one to the other the presence of giant cholesterol bearing molecules in the serum and the tendency of the individual to develop intramural arterial hemorrhages

Clinical pathological and experimental data support the contention that there is a relationship between the degree of arteriosclerosis and the general state of bodily nutrition The usual candidates for coronary artery disease are those that are well nourished The high incidence of the fatal sequelae of coronary arteriosclerosis in the obese has been known to life insurance statisticians for years [13] Wilens [60] in a large series of autopsies on elderly persons found a marked reduction in the severity of arteriosclerosis of the atherosclerotic type in individuals who were undernourished compared with those who were either in a normal state of nutrition or were frankly obese Comparable results have been obtained in experimental animals Although the feeding of cholesterol to rabbits results in arterial lesions which roughly parallel the degree of induced hypercholesterolemia the weight of the animal seems to be of importance in governing the severity of the disease Pollak[61] found the more severe experimental lesions in his heavier and older cholesterol fed rabbits Firstbrook[20] has attacked the problem using a statistical approach Cholesterol was given to some rabbits which were fed *ad libitum* and to others which received only 50 per cent of the amount of food consumed by the animals in the first group On comparing the degree of arterial disease in the two groups a high net correlation between relative weight gain and severity of experimental lesions was found Additional evidence has been obtained in other species that the accumulation of lipids in arterial walls may be reversible When cholesterol is fed to chickens lipids are deposited in pre existent spontaneous fibrous plaques of the intima of the coronary arteries but when the birds are returned to a normal diet, without added cholesterol the plaques lose most of their lipid and revert to their usual fibrous appearance [50] A similar resorption of lipids has been noted in the experimental cholesterol lesions of dogs [2] Much more work is needed along these lines but at the moment the evidence supports the contention that overnutrition favors the acceleration of the arteriosclerotic process and there is therefore some justification for advising middle aged individuals to keep in shape Lipotropic agents like choline to prevent the deposition of lipids in arterial walls or to assist in their removal have been advocated in cases of proven coronary artery disease [40] but further controlled clinical trials are needed before the use of these agents is justified

Elevation of the blood cholesterol level has been considered for years to be a major factor in the initiation, or the acceleration of the arteriosclerotic process The increased incidence of the more serious manifestations of the disease in persons with familial hypercholesterolemia [5,59] in those with an elevated blood lipid or blood cholesterol level due to diabetes mellitus the nephrotic syndrome and hypothyroidism and the decreased incidence of arteriosclerosis in those with depressed blood cholesterol levels from hyperthyroidism have strengthened the position of the proponents of the hypercholesterolemic theory The production of experimental arteriosclerosis in certain animal species by cholesterol feeding

The *capillary vascularization* theory of Winternitz has been abandoned. It postulates an initial rupture of capillaries in the intima of normal arteries, but it is now agreed that intimal vascularization is a result, rather than a cause of arteriosclerosis.

The combined *lipophage vascularization* theory, proposed by Katz and Dauber suggests that migratory, blood borne foam cells enter the orifices of intimal capillaries obstruct these fine channels, and result in necrosis and cholesterol deposition. This theory is open to the same objections which have been made to that of Winternitz and of Leary.

Recently the *thrombotic* theory has been revived by Duguid. By it the initial lesion is a small mural thrombus which for unspecified reasons, is laid down upon the intact endothelium and from this thrombus the first cholesterol deposits are derived. This theory lacks morphological confirmation in man and in experimental animals.

On the whole the theory in most favor at the moment is that the plasma lipid mixture normally permeates the inner portions of the arterial wall by a process of non selective infiltration, that instability of serum cholesterol predisposes to its deposition and that it is deposited only at points of pre existent vascular injury. Without the primary injury then arteriosclerosis of the atheromatous type should not occur and without the instability of serum cholesterol the disease should not progress as a rule to serious proportions. This combined theory of injury and imbibition will now be examined in some detail.

The hypothetical injurious agent which produces the equally hypothetical primary vascular injury in man has received little attention. But an injury must be postulated as otherwise the disease process would be diffusely spread throughout the arterial system instead of being local in its distribution. The hypothesis in most favor at the moment is that simple mechanical factors govern the localization of lesions that the plasma lipid mixture is laid down at points where the arterial wall is altered or injured by stress. Another hypothesis has been suggested by Dock[1] to explain the localization of lesions. The intima of the coronary arteries of infants is much thicker than is the same layer in other arteries which are not so susceptible to arteriosclerosis, the intimal thickening is 3 times greater in male than in female infants and it is focal in its distribution. Dock suggests that these congenital fibrous thickenings are the sites for the subsequent deposition of lipids and since they are larger in males than in females the sex difference in the incidence of coronary artery disease is thus explained.

The findings in experimental animals suggest a different type of primary injury. Rabbits which develop atheromatous deposits in their arteries in response to cholesterol feeding show a primary degenerative lesion of the medial coat and this primary lesion is due presumably to some noxious agent inherent in the technique of cholesterol feeding.[14] The primary arterial injury in the chicken is also located in the media but as mentioned in the previous section it is due to an infectious process which is peculiar to the avian family and is completely unrelated to cholesterol feeding.

Actually the cause of the initial lesion in human arteriosclerosis is of academic interest. Hardly anyone over the age of forty years escapes some stigmata of the disease but because the lesions are often so slight no symptoms result. On the other hand a certain number of individuals show a marked acceleration of the disease process and this acceleration is not confined to the older age groups. Here

then is the problem of practical importance why do the lesions in some individuals progress to the point that they produce symptoms while in others they remain small and innocuous? No fixed rules can be offered, but from the evidence at hand it would appear that the following factors play some part in the progression of the arteriosclerotic process—the general state of bodily nutrition the blood lipid level the ratio of blood lipids one to the other, the presence of giant cholesterol bearing molecules in the serum and the tendency of the individual to develop intramural arterial hemorrhages

Clinical, pathological and experimental data support the contention that there is a relationship between the degree of arteriosclerosis and the general state of bodily nutrition The usual candidates for coronary artery disease are those that are well nourished The high incidence of the fatal sequelae of coronary arteriosclerosis in the obese has been known to life insurance statisticians for years [13] Wilens,[60] in a large series of autopsies on elderly persons found a marked reduction in the severity of arteriosclerosis of the atherosclerotic type in individuals who were undernourished compared with those who were either in a normal state of nutrition or were frankly obese Comparable results have been obtained in experimental animals Although the feeding of cholesterol to rabbits results in arterial lesions which roughly parallel the degree of induced hypercholesterolemia, the weight of the animal seems to be of importance in governing the severity of the disease Pollak[31] found the more severe experimental lesions in his heavier and older cholesterol fed rabbits Firstbrook[20] has attacked the problem using a statistical approach Cholesterol was given to some rabbits which were fed *ad libitum* and to others which received only 50 per cent of the amount of food consumed by the animals in the first group On comparing the degree of arterial disease in the two groups a high net correlation between relative weight gain and severity of experimental lesions was found Additional evidence has been obtained in other species that the accumulation of lipids in arterial walls may be reversible When cholesterol is fed to chickens lipids are deposited in pre existent spontaneous fibrous plaques of the intima of the coronary arteries but when the birds are returned to a normal diet without added cholesterol the plaques lose most of their lipid and revert to their usual fibrous appearance [50] A similar resorption of lipids has been noted in the experimental cholesterol lesions of dogs [2] Much more work is needed along these lines but at the moment the evidence supports the contention that overnutrition favors the acceleration of the arteriosclerotic process and there is therefore some justification for advising middle aged individuals to keep in shape Lipotropic agents like choline to prevent the deposition of lipids in arterial walls or to assist in their removal have been advocated in cases of proven coronary artery disease [40] but further controlled clinical trials are needed before the use of these agents is justified

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techniques has added further weight to their argument. The rabbit is particularly susceptible to this procedure, and recently the combination of thiouracil with cholesterol feeding has succeeded in producing arterial lesions in the dog which are remarkably like those in man.[54] In the dog the plaques even show vascularization and intimal hemorrhage. The chicken has also been cited as an example of the hypercholesterolemic origin of arteriosclerosis,[10, 11] but other workers have shown that cholesterol feeding in birds results only in an acceleration of arterial lesions which are initiated by another mechanism.[47]

However, a major inconsistency in the hypercholesterolemic theory has been the failure to demonstrate elevated blood cholesterol levels in many individuals who are obviously suffering from end stage coronary artery disease. Recent developments in the field of lipid chemistry have suggested reasons for this discrepancy. The ground was laid by the interesting studies of Duff and his co workers on the development of arteriosclerosis in alloxan diabetic rabbits. They compared the effects of cholesterol feeding in normal rabbits and in rabbits rendered persistently diabetic by means of alloxan. Comparable degrees of hypercholesterolemia were induced in the two groups of animals, but the severity of arteriosclerosis in the diabetic rabbits was much less than in the non diabetic control animals. Indeed a large proportion of the diabetic animals presented no arteriosclerosis whatever.[15] From this experiment, which has been confirmed,[39] it is apparent that hypercholesterolemia *per se* is not responsible for the deposition of lipids in the arterial walls of cholesterol fed rabbits. Later experiments have shown that the governing factor lies, rather, in the stability of serum lipids. Provided that there is a proportionate elevation of serum lipid phosphorus and neutral fat, a marked hypercholesterolemia can exist in the rabbit without the development of arteriosclerosis.[16] The importance of the cholesterol phospholipid ratio has been demonstrated in another way by Kellner and his collaborators.[23] By injecting synthetic detergents intravenously into cholesterol fed rabbits they achieved an elevation of serum cholesterol which was accompanied by a parallel rise of phospholipids, and in these animals the development of arterial lesions was inhibited compared with that in control cholesterol fed rabbits which showed a rise in blood cholesterol without a concomitant rise of phospholipids.

Another important observation on the role of cholesterol and other serum lipids in the pathogenesis of arteriosclerosis has been made recently by Gofman and his co workers.[20] Using ultracentrifugal methods they have found that the presence of giant cholesterol bearing molecules in the serum can be related to the pathogenesis of arteriosclerosis in the experimental animal and in man. These giant molecules are present in higher concentrations and at a much higher frequency in patients who have survived myocardial infarction than in those without known vascular disease. The blood level of the molecules can be closely correlated with those human diseases which are usually associated with an excessive amount of arteriosclerosis and with experimental cholesterol atherosclerosis in the rabbit. It is poorly correlated with the analytical serum cholesterol level. Most important of all, the blood level of the molecules can be influenced by diet.

These observations on the cholesterol phospholipid ratio and on the presence of giant cholesterol bearing molecules in the serum may well have a bearing on the pathogenesis of the human disease, but to establish or refute this relationship is difficult. The stumbling block lies in the fact that the diagnosis of human arteriosclerosis of any degree is often impossible except on the autopsy table. The

only reasonably reliable clinical manifestations are the signs and symptoms of coronary artery disease, but even here there are fallacies. It is fair to assume that patients with demonstrable cardiac infarcts are probably suffering from a moderate or severe degree of coronary arteriosclerosis, but it does not follow that men without cardiac infarcts do not harbor coronary arteriosclerosis of a similar degree. Uncomplicated progressive coronary arteriosclerosis, leading to marked stenosis of major coronary branches, does not necessarily result in symptoms, since it is compensated for by the opening up of a collateral circulation which is sufficient for the ordinary demands of life.[4] Furthermore, the production of coronary occlusion with myocardial infarction appears to result from factors which often have no relationship to the level or character of the blood lipids at the time of the catastrophe, and these extraneous factors will be discussed in the next section. Thus to establish a relationship between disorders of the serum lipids and an acceleration of the arteriosclerotic process, a more reliable method of assessment is needed. In the opinion of the writer, this can only be done by comparing serial determinations of the serum lipids during life with an accurate estimation of the degree of arteriosclerosis as seen at autopsy. In carrying out an assessment of this type, two points must be borne in mind. Since arteriosclerosis is a disease which may take years to develop and may persist for some time after the causative agent has ceased to act, the serial determinations of blood lipids should be made over long periods. And it would be helpful if a technique could be devised for estimating the degree of disease at autopsy which is more accurate than the crude methods of grading in use today.

Finally, acceleration of the arteriosclerotic process may be produced by a mechanism which is quite distinct from the biochemical alterations just described—by repeated bouts of hemorrhage into pre-existent arteriosclerotic plaques. Winternitz and his co-workers[61, 6] have been so impressed with the frequency of hemorrhages into the intimal layers of arteries that they have suggested that they not only accelerate the arteriosclerotic process but may actually initiate it. Intimal hemorrhage cannot be admitted as a causative lesion in arteriosclerosis for reasons given previously in this chapter, but as an accelerating factor it deserves consideration. In my experience, intimal hemorrhages are extremely common in association with moderate or severe grades of coronary arteriosclerosis. They can be seen with the naked eye when the artery is opened, when they appear as blotchy, slightly elevated, red or brownish black discolorations (Figure 38); they are often multiple in individual cases and are especially common in hypertensive individuals. Although they were formerly considered to be the result of backflow of blood from the lumen through an intimal defect, serial section technique has revealed that often there is no break in the tissues lying between the hemorrhage and the lumen of the artery.[4, 43] These intrinsic hemorrhages are clearly the result of rupture of the newly formed intimal capillaries which are such a common feature of the arteriosclerotic process.[42, 6]

Intimal hemorrhage may produce a variety of sequelae, as will be described later, but one effect must be an increase in size of the affected plaque. The enlargement will be due at first to the addition of blood to the bulk of the plaque and later to the presence of the products of the breakdown of blood and to the reaction of the tissues to these products. The apparent increase in size of arteriosclerotic plaques into which hemorrhages have occurred is illustrated in Figures 39 and 40. Single hemorrhagic episodes like these would produce little effect



FIG 38 —*Intimal hemorrhage of a coronary artery* as seen on gross examination with the artery opened

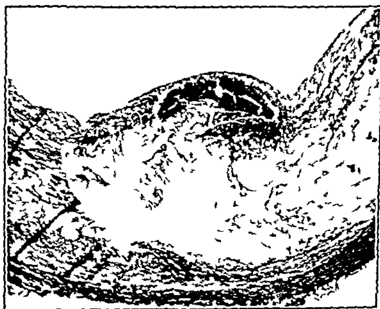


FIG 39 —*Intimal hemorrhage without thrombosis of a coronary artery* The hemorrhage appears to have enlarged the plaque slightly

once the blood was absorbed, but if the process were repeated frequently into the same plaques a gradual increase in their bulk should occur In this regard, it is to be noted that many arteriosclerotic plaques contain blood elements in varying stages of dissolution, indicating the repetitive nature of intramural hemorrhage

It would be wrong to attribute all, or even most, of the acceleration of the arteriosclerotic process in man to these hemorrhagic episodes although no doubt they may play some part especially in association with certain disease processes As will be discussed in the next section, one of the hypothetical factors which may produce rupture of intimal capillaries is high intracapillary pressure from arterial hypertension and this may explain, in part, why severe arteriosclerosis is so common in hypertensive individuals

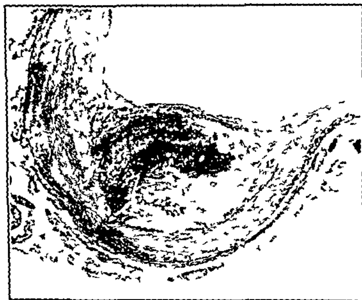


Fig 40 — *Intimal hemorrhage of moderate size into an arteriosclerotic plaque of a coronary artery* Thrombosis has not resulted

THE MECHANISM OF CORONARY OCCLUSION

Myocardial ischemia so severe that sudden death occurs or so prolonged that irreversible changes are set up in a large segment of the cardiac musculature may result from a variety of causes Some of these like coronary embolism are rare — too rare to be discussed here The common mechanisms are the ones which result directly from coronary arteriosclerosis and the present section will be limited to the manner in which these mechanisms operate and to their clinical implications

Acute functional insufficiency of the coronary circulation from a sudden increase in the nutritional demands of the heart in the presence of severe arteriosclerotic stenosis is far more common than is generally realized Indeed as a cause of sudden death from heart attacks and as a cause of myocardial infarction it ranks in frequency with coronary thrombosis[63] The underlying lesion is uncomplicated marked narrowing of one or more major coronary branches and

the trigger mechanism is a sudden call for more blood than can be supplied through the stenosed arterial lumina. The increase in heart work which occurs with excessive physical exercise or emotional stress is therefore a major precipitating factor, although the same mechanism may operate when the blood pressure drops suddenly from shock or if profuse hemorrhage depletes the oxygen carrying capacity of the blood.

The prevention of acute coronary insufficiency should be directed against each of the factors involved, and should include the restriction of physical activity and the avoidance of emotional stress in individuals of advancing years, a dietary regimen which will not only correct obesity and thus decrease heart work but which may also reverse to a degree the lipid deposits in the arterial wall, the prevention of shock and the replacement of blood lost in surgical operations, and attempts to aid the development of an efficient collateral circulation by medical or surgical means. These are some of the measures which now lie at our disposal, and when we add to them the possibility that regression of the arteriosclerotic process may some day be achieved by specific therapeutic agents, the ultimate prognosis for the end stage uncomplicated arteriosclerotic individual is by no means hopeless.

It is to be particularly noted that the precipitating factor of myocardial ischemia in acute coronary insufficiency lies outside the coronary circulation. In it the degree of arterial stenosis is no greater with the onset of symptoms than it was before. In the conditions to be described now, the reverse is the case—the disproportion between supply and demand results from secondary lesions within the arteries proper. Acceleration of the stenosing effect of an arteriosclerotic plaque to the point of occlusion of the lumen of the artery, may be accomplished in either of two ways: (1) by the sudden enlargement of the plaque from a massive hematoma, and (2) by obliteration of the lumen by a thrombus. Each of these occluding lesions has a common mechanism and they will be discussed together.

The etiology of bland (non infective) arterial thrombosis has received a good deal of attention in recent years. It is generally agreed that arteriosclerosis, of a significant degree, is the essential underlying lesion, and from it arise the two factors which are needed for the initiation and propagation of a thrombus. One of these is supplied by the shape and size of the arteriosclerotic plaque which by projecting into the arterial lumen results in the critical amount of stasis and eddying of blood for thrombus propagation. The other, the lesion which liberates thromboplastic substances and thus initiates the thrombus, develops within the body of the plaque. Three initiating lesions have been described—an inflammatory focus by Boyd,[6] an ulcerated surface by Leary[29] and a disruptive intimal hemorrhage by the writer.[42, 43] There are reasons to believe that these three lesions are part and parcel of the same fundamental process.

The striking changes that occur in the blood supply to the wall of an artery which is thickened by arteriosclerosis have been described in a previous section. Newly formed capillaries grow into the intima from the lumen of the artery, they penetrate the outer shell of dense fibrous tissue and ramify about the mass of atheromatous debris in the core of the plaque (Figs. 37 and 38). Intimal capillaries may and do rupture and intramural hematomata of varying sizes and with varying results are formed.

Of the various sequelae of intimal hemorrhages of arteriosclerotic coronary arteries, that of thrombus precipitation appears to be of the most importance (Figs. 41 and 42). As first described, the sequence of events is as follows:

1 Intimal capillaries rupture a hematoma forms and this is accompanied by the disruption of tissue

2 Thromboplastic substances are liberated from the area of hemorrhage in one or more of the following ways when the hemorrhage is superficial there may be diffusion of these substances through the intact intima into the main arterial lumen The rupture of nutrient capillaries may be so extensive that necrosis of the intima results thus forming an atheromatous ulcer with a raw surface When the hemorrhage occurs into the deeper intimal layers the capillaries adjacent to



FIG 41 —*Intimal hemorrhage and thrombosis of a coronary artery* The thrombus is deposited in close relationship to the hemorrhage



FIG 42 —*Intimal hemorrhage and thrombosis of a coronary artery*

the point of rupture may thrombose, retrograde capillary thrombosis may then take place and when the process reaches the mouth of the capillary it may form the nucleus for the occluding mass in the arterial lumen

3 A coronary thrombus forms at the site of damage but only if suitable conditions of stasis and eddying of blood exist

If it be true that intimal capillaries are a compensatory device for the nutrition of an abnormally thickened intima their rupture will interfere with the necessary blood supply and necrosis of the intima with the formation of an ' atheromatous ulcer ' should be the result In other words atheromatous ulceration as a cause of thrombus precipitation as described by Leary,[29] is in effect a sequel of capillary rupture, just as much as is an intimal hematoma And when inflammatory foci develop in arteriosclerotic plaques and result in coronary thrombi, as described by Boyd [6] one can usually demonstrate hemorrhage as well as inflammatory infiltration at the point of initial thrombosis It would appear that both inflammation and hemorrhage are the result of capillary rupture, the inflammatory cells being a reaction to the presence of free blood or of necrotic tissue

The hypothesis that intimal hemorrhages are concerned in the precipitation of coronary thrombi is gradually gaining acceptance Since 1936 when it was first suggested,[4] it has had ample confirmation by Wartman [5] Winternitz and his co workers,[8] Horn and Finkelstein [27] and Nelson [41] Similar lesions may initiate *thrombosis in arteriosclerotic arteries in other parts of the body*—in cerebral arteries[45] and in arteries of the lower extremity [3] [57] In spite of the unanimity of opinion expressed by the above mentioned workers, however, a certain amount of scepticism still exists

It has been suggested for example that a hemorrhage which lies at the point of thrombotic occlusion may be the result rather than the cause of thrombosis that it represents a type of hemorrhagic infarction of the vessel wall at a point where it has been deprived of its blood supply by the occluding mass This possibility is effectively eliminated by the fact that intimal hemorrhages can be demonstrated repeatedly at points where there is no associated thrombosis Discrete lesions of this type are illustrated in Figures 39 40 and 41

Conversely because intimal hemorrhages occur so often without thrombosis it has been suggested that their association with thrombi is purely coincidental This argument is difficult to refute However *because as many as 90 per cent of* coronary thrombi are deposited at points of intimal hemorrhage (Table VI) and because careful serial section will often show the oldest part of the thrombus, or a partially occluding thrombus to lie in close proximity to the hemorrhage (as illustrated in Figures 42 and 43) the factor of coincidence appears to be improbable

TABLE VI —INCIDENCE OF INTIMAL HEMORRHAGE IN CORONARY THROMBOSIS USING DIFFERENT PATHOLOGICAL TECHNIQUES

Investigator	*Pathological Technique*	*Cases of Thrombosis Studied*	*Intimal Hemorrhages*	
			Number	*Per Cent*
Paterson[44]	Serial section at short interval	58	52	89
Horn and Finkelstein[27]	Many section	123	64	52
Nelson[41]	Many section	10	9	90
Paterson	Many section	34	21	62
Yater *et al*	Occasional sections	158 (?)	26 (?)	16

Scepticism still exists on another score while it is conceded that an intimal hemorrhage may cause thrombosis in certain cases, its frequency as a precipitating lesion has not been considered by some to be impressive Here again some fairly exact data can be quoted From a consideration of the evidence given in Table VI it would appear that the incidence of hemorrhages in any series of cases of coronary thrombosis depends entirely upon the pains that are taken in searching for them When thrombosed segments of arteries are sectioned serially at close intervals the incidence is as high as 90 per cent when the interval between sections is greater but many sections are examined the incidence is approximately 60 per cent while if only occasional random sections are studied the incidence falls to less than 20 per cent The variation between these results can be easily explained Intimal hemorrhages are often quite small and these smaller lesions can only be demonstrated by serial section at fairly close intervals If they were usually large and easily demonstrable by random sections I suspect that they would have been observed and described long before 1936

From the foregoing it appears certain that intimal hemorrhage, or other sequelae of capillary rupture is the usual precipitating lesion in coronary thrombosis In the final analysis then, the factors responsible for the rupture of intimal capillaries are the immediate causes of precipitation of most coronary thrombi These factors are admittedly highly debatable They will probably not be definitely established until they can be studied under controlled experimental conditions and studies of this type must await the production of end stage coronary arteriosclerosis with vascularized plaques in susceptible animals In the meantime there are two schools of thought First there are those like Master[3] who regard capillary rupture with intimal hemorrhage as a purely fortuitous event in the course of arteriosclerosis, who claim that it therefore cannot be predicted prevented or treated The second school has a more optimistic outlook, and to it the writer strongly subscribes It holds that capillaries in arteriosclerotic plaques rupture from certain definite causes and that these should be sought for industriously Some of these hypothetical factors have already been suggested[4, 46] and they will be reviewed here briefly

Intracapillary pressure appears to play a major role in the rupture of capillaries in arteriosclerotic plaques Intimal capillaries like the one shown in Figure 37 are peculiar in that they lie in direct communication with the lumen of a large artery in which the pressure of blood even if normal is relatively high approximating that in the ascending aorta They are not like ordinary capillaries at the end of a long series of arteries and arterioles which absorb much of the pressure by friction It is reasonable to assume that the pressure in intimal capillaries of the coronary arteries will be further increased in cases of persistent hypertension or of temporary elevation of blood pressure from excessive exertion or emotion The effect of exertion and emotion upon the arterial blood pressure is a very real one With strenuous muscular exercise the systolic blood pressure is said to rise to from 160 to 180 mm of mercury A corresponding rise in the diastolic pressure although to a lesser degree also occurs Emotional stress often but not always, results in a similar sudden increase in the systolic and diastolic pressures For example a case is on record[23] of a marked increase in the blood pressure of a young woman each time she recounted the details of a particularly harrowing experience This woman showed a normal variation in blood pressure of from 120 to 140 mm

systolic and from 80 to 95 mm diastolic During an emotional upset the systolic pressure varied from 150 to 180 mm and the diastolic from 100 to 120 mm

On purely theoretical grounds then, one would expect that hypertension persistent or transient should increase the strain upon the walls of intimal capillaries and there should be imminent danger of capillary dilatation and rupture The pathological evidence supports this assumption Coronary thrombosis is more common in hypertensive than in non hypertensive cases [12 36] Furthermore, I have analysed a series of elderly individuals with similar grades of coronary arteriosclerosis and have found that intimal hemorrhages with and without thrombi are much more numerous in those with persistent hypertension than in those with normal blood pressures [46]

Intimal hemorrhages, therefore, do not appear to be merely fortuitous events in the course of the arteriosclerotic process Other conditions being equal they are more apt to occur in hypertensive than in non hypertensive individuals From this it may be deduced that transient hypertension from emotional stress or excessive physical exercise will have the same effect it will favor capillary rupture to a degree and produce similar lesions This deduction has an obvious medico legal application which will be discussed in a later section of this chapter

Before leaving the subject of intracapillary pressure, it should be noted that in one respect chance may play a part in the rupture of capillaries in arteriosclerotic plaques From a consideration of Bernouille s Principle it has been suggested[8] that the pressure within an intimal capillary will vary with the site of its origin from the arterial lumen If a blind capillary arises at the apex of a stenosing arteriosclerotic plaque where the blood pressure is low but the blood flow fast the chance of rupture will be less than if it arises at the edge of the plaque where the pressure is high but the blood flow slow But with either of these positions of origin the intracapillary pressure should be appreciably greater in hypertensive than in non hypertensive individuals

Other factors besides intracapillary pressure may play a part in the rupture of intimal capillaries and in the initiation of coronary thrombi Increased capillary fragility is an obvious cause for capillary rupture and this may arise in various ways—from local inflammatory conditions in the vessel wall from toxic influences of a more general nature from advancing age and from inadequate nutrition Some of these factors cannot be corrected but inadequate nutrition can In this regard one thinks immediately of avitaminosis C The mode of action of vitamin C is not known with certainty but the essential pathological change appears to be a weakening of the capillary endothelium from a reduction in the amount of intercellular cement substance On theoretical grounds avitaminosis C should thus be a possible cause of intimal hemorrhages and there is some evidence that it is not infrequent in cases of coronary artery disease [46] However the whole field of vitamin C nutrition is still in its infancy and much more experimental and clinical evidence is needed before any definite claims are warranted In the meantime the recommendation that patients with coronary artery disease be assured of an adequate vitamin C intake would appear to be justified

The rigidity of the tissues which surround intimal capillaries may also play some part in preserving their integrity Intimal hemorrhages occur almost exclusively at points of extreme atheromatous degeneration of arteries at points of ' softening rather than of hardening Presumably the lack of rigidity in atheromatous foci allows the pressure of blood within a capillary to dilate its wall to the

point that rupture eventually occurs This assumption is supported by the fact that younger individuals with characteristically dense and fibrous arteriosclerotic plaques and elderly persons with heavily calcified lesions are not so prone to intimal hemorrhages and coronary thrombi as are those in the middle age group in whom atheroma is a striking feature The calcification of arteries has been described as but another manifestation of the general principle that any area of devitalized tissue will become infiltrated with lime salts[58] The calcification of arteriosclerotic arteries may thus be a protective phenomenon strengthening weakened areas and perhaps preventing capillary rupture But whether or not these theoretical considerations will lead to a therapeutic approach to the prevention of capillary rupture and coronary thrombosis remains to be seen

Up to this point the importance of thrombosis as a cause of coronary occlusion has been stressed However another occlusive mechanism by a large intimal hematoma without associated thrombosis[55,56] is now recognized Occasionally, these hematomata do reach massive proportions and it is reasonable to assume that they may so stenose the lumen of the artery that sudden death or myocardial infarction may result At the same time, the caliber of the arterial lumen in these cases prior to capillary rupture cannot be estimated, and thus some of the stenosing effect of intimal hemorrhage may be more apparent than real[43] In any event my experience has been that coronary occlusion by a massive hematoma *per se* is decidedly rare it accounts for less than 10 per cent of the occlusions found in a large series of cases which have been studied by serial section technique In two respects however massive hematomata have interesting clinical implications Because they represent a sudden and massive hemorrhage into an arteriosclerotic plaque the interval of time between the primary capillary rupture and the resultant myocardial ischemia must necessarily be short And this has a medico legal application which will be discussed in a subsequent section

Of more importance is the possibility that an intimal hemorrhage may assume serious proportions in a patient on anticoagulant therapy[4] This danger seems to have been grossly exaggerated I have recently had an opportunity of studying the coronary arteries of 8 patients who succumbed from coronary artery disease at a time when they were receiving Dicumarol Recently formed intimal hemorrhages were noted in each case but in none were they of large size Indeed they were indistinguishable in their bulk from those seen routinely in autopsies on patients who had not received anticoagulants One of the largest of the hemorrhages in the patients who had received Dicumarol is illustrated in Figure 39 Intimal hemorrhages which precede and cause the deposition of coronary thrombi should be even less apt to assume serious proportions in patients who subsequently receive anticoagulants By the time the drug is given (with the onset of cardiac pain) all danger of excessive bleeding into an arteriosclerotic plaque should be past

This section would not be complete without some mention of the possible benefits to be obtained from anticoagulant therapy in patients with recent myocardial infarcts There seems to be no question that a reduction in mortality can be achieved by this procedure which prevents the development of thrombo-embolic complications[63] However these good results have only been obtained under conditions of close supervision conditions which are apparently not practicable in ordinary hospital practice In this regard a group of workers at the Boston City Hospital have reported their failure to obtain a reduction in mortality in

patients who received Dicumarol therapy in their hospital[7] What seems to be needed is a new anticoagulant, the dosage of which can be controlled by the simplest of laboratory procedures, and which is safer, more efficient and less expensive than Dicumarol

PREMONITORY SYMPTOMS OF CORONARY THROMBOSIS

Although it has been recognized for some time that an attack of coronary occlusion may be preceded by warning symptoms, remarkably little interest has been displayed in the mechanism of production of these symptoms, in their clinical identification and in their therapeutic implications The lack of interest stems in part from the fact that only some of the cases of coronary occlusion have premonitory symptoms and in part from the highly indefinite nature of the symptoms themselves

A ready explanation is at hand to account for the absence of premonitory symptoms in some cases In two of the three major varieties of occlusion the sequence of events is so fast that there is no time for warning symptoms to be recognized In acute coronary insufficiency the sudden urgent demand for more blood through the inadequate coronary circulation produces an effect that is practically instantaneous Occlusion by a massive intimal hematoma should also develop rapidly within *the time that it takes blood to clot under ordinary conditions* and again no premonitory symptoms would be expected On the other hand evidence is gradually accumulating that hours or days may elapse between the time of inception of a coronary thrombus and the moment when occlusion, with its resultant cardiac pain occurs During this interval of time when a thrombus is growing by accretion symptoms might well be produced

The literature on the speed of formation of coronary thrombi is recent and quite scant In 1935 Barnes[1] stated that "occasionally coronary thrombosis occurs gradually the vessel not becoming completely occluded for from one to three days In the following year Clark and his co workers[9] demonstrated the slowly progressive character of a thrombus in 1 out of 11 consecutive cases However since that time evidence has been obtained that slowly produced thrombi are the rule rather than the exception Levy and Bruen[30 31] found that the appearance of the occluding thrombi in many cases of sudden death indicated that they had formed hours or days before the heart had ceased to beat I have obtained additional information by sectioning serially the entire length of coronary thrombi from individuals who died suddenly and in whom infarction had not had time to take place In 5 consecutive cases studied in this way the thrombotic process was hours or days in age at its point of inception (as determined by its staining qualities or by its invasion by fibroblasts) while it was of a recent nature where it occluded the arterial lumen[41 49] Photomicrographs of two slowly growing coronary thrombi are shown in Figures 43 and 44 and in the latter there are four distinct strata suggesting that precipitation had occurred in waves rather than continuously From this type of evidence it would seem that most coronary thrombi are produced slowly and that when they reach a certain size they may result in premonitory symptoms

A major difficulty lies in the recognition of premonitory symptoms Many of them are far from diagnostic they may simulate symptoms of other diseases to say nothing of their possible neurotic origin Feil[19] reported 15 cases of coronary

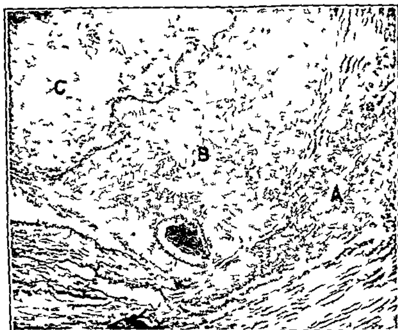


FIG 43 —*A coronary thrombus which has been laid down in two distinct layers* A the superficial intimal tissue into which hemorrhage had occurred some time previously B the initial thrombus deposition showing invasion by fibroblasts C recent thrombus which occludes the lumen of the artery



FIG 44 —*A coronary thrombus showing four distinct strata of thrombus deposition* (at B C D and E) A the inner portion of the intima

thrombosis which had preliminary mild anginal attacks preceding the clinical pitcure of occlusion by hours or days, usually twelve to forty eight hours The pain was not dependent on effort or emotion It was more or less continuous and of a burning and oppressive character A gradually forming thrombus appeared to Feil to be the most probable explanation for these preliminary symptoms Master, Dack and Jaffe[37] found that premonitory symptoms were present in 44 per cent of 260 patients with acute coronary occlusion In most cases they consisted of substernal or precordial pain or discomfort Other prodromal symptoms were fatigue weakness gastric distress, dyspnoea palpitation, nervousness and dizziness *The sudden appearance of an atypical anginal syndrome frequently* preceded the attack of occlusion The premonitory symptoms usually appeared within twenty four hours of occlusion, and they lasted for varying periods—from a few minutes to several hours While the symptoms were usually more or less continuous a pain free period sometimes supervened before the onset of the syndrome of acute occlusion The anginal pain experienced by these patients was not influenced by nitroglycerin as it is in typical angina pectoris and this has been noted by others

The evidence at hand suggests strongly that the premonitory symptoms described by Feil and by Master and his co workers are due to partial closure of a major coronary artery by a gradually forming thrombus Unfortunately the symptoms as described are far from specific, and there is an urgent need for the development of a clear cut syndrome by which this stage of incipient thrombotic occlusion might be recognized If it could be recognized consistently therapy might be directed along at least two lines—anticoagulant and antispasmodic

It is obvious that if the stage of partial closure of a coronary artery by a gradually forming thrombus could be diagnosed emergency anticoagulant therapy might prevent further thrombus precipitation and a threatening terminal occlusion might thus be aborted Another possible therapeutic approach is suggested by the experimental studies of Manning and his co workers[23 34 38] They have shown that ventricular fibrillation with generalized myocardial ischemia and sudden death often occurs in experimental animals when a major coronary artery is ligated and that this is probably due to a generalized reflex coronary spasm which is initiated by the occlusion of the artery They have shown further that this reflex spasm and sudden death can be prevented to a degree by bilateral cardiac sympathectomy by sympathetic inhibiting agents and by certain coronary antispasmodic and vasodilator drugs Of these latter agents it is of interest to note that intravenous ethyl alcohol administration is by no means the least effective Thus if premonitory symptoms of coronary thrombosis could be recognized emergency therapy along some of these lines might prevent reflex coronary spasm and sudden death if and when terminal occlusion occurs

In the foregoing the assumption has been made that the so called premonitory symptoms *of coronary thrombosis are due to incomplete closure of an artery by a* gradually forming thrombus However it should be mentioned that another mechanism has been suggested that the symptoms are due to the irritation of afferent nerve endings in the arterial wall from the tension produced by an intimal hemorrhage[4] This mechanism appears improbable for two reasons nerve endings have never been demonstrated in the intimal layer of arteriosclerotic arteries and evidence has been obtained that patients who show ordinary discrete intimal hemorrhages of their coronary arteries at autopsy without asso

ciated thrombi, have not complained of symptoms of the premonitory variety during life [4]

THE RELATION OF STRESS TO CORONARY OCCLUSION

The possible relationship of excessive physical exertion or emotion to the production of coronary occlusion has recently been the subject of widespread debate and conjecture The situation has been confused by the results of purely clinical studies dealing with the activities of patients at the time they develop the syndrome of occlusion Because many patients are at rest or asleep at the time of onset of cardiac pain it has been argued that excessive exertion or emotion is not a factor in the production of coronary occlusion [35] These claims, however are based on the assumption that all occluding lesions of the coronary arteries are initiated and produce their full occluding effect within a very short space of time—an assumption which is not true for coronary thrombi [44] The subject has important medico legal applications particularly in Workmen s Compensation Board cases and an attempt will be made in this section to clarify the position from the standpoint of the pathologist The crux of the matter may be stated in the following hypothetical case

A sixty year old man apparently in good health and employed in a sedentary type of work is ordered by his employer to do a job which called for an unusual and for him an exhausting expenditure of energy He does the work and at its completion he complains of severe chest pain collapses and dies within a few minutes The question is was his death the direct result of his unusual physical exertion or not?

An autopsy is carried out the cause of death is laid to coronary artery disease, and depending on the lesions noted the following views in my opinion may be expressed

If the coronary arteries show an extreme grade of arteriosclerotic stenosis *but no occluding lesion is found* death can be attributed to acute coronary insufficiency the direct result of the unusual physical exertion This opinion is supported by the authoritative anatomical studies of Blumgart Schlesinger and Davis[4] who have shown that arteriosclerotic stenosis of the coronary arteries is compensated for by the development of a collateral circulation and is not inconsistent with an efficient blood supply to the heart under ordinary conditions of life However if the nutritional demands of the myocardium exceed this normal level as they may with the increased heart work which results from severe exertion an imbalance may be created which leads to myocardial ischemia In my opinion this mechanism is beyond all argument

If autopsy reveals that rather rare lesion a massive intramural hematoma which occludes the lumen of a major coronary artery death can again be laid to the unusual physical exertion This opinion is based on indirect evidence but it seems to have been accepted by most workers in the field [41 42 55] The argument runs as follows excessive physical exertion usually results in a transient rise in blood pressure the increased arterial pressure is reflected into intimal capillaries of an arteriosclerotic plaque the capillaries dilate and rupture and a massive intramural hematoma is formed Because the formation of the hematoma is probably rapid within the time that it takes blood to clot the interval between capillary rupture and coronary occlusion should be short and thus consistent with the time relationships in the case under discussion

thrombosis which had preliminary mild anginal attacks preceding the clinical pitcure of occlusion by hours or days, usually twelve to forty eight hours The pain was not dependent on effort or emotion It was more or less continuous and of a burning and oppressive character A gradually forming thrombus appeared to Feil to be the most probable explanation for these preliminary symptoms Master, Dack and Jaffe[37] found that premonitory symptoms were present in 44 per cent of 260 patients with acute coronary occlusion In most cases they con sisted of substernal or precordial pain or discomfort Other prodromal symptoms were fatigue weakness gastric distress dyspnoea, palpitation nervousness and dizziness The sudden appearance of an atypical anginal syndrome frequently preceded the attack of occlusion The premonitory symptoms usually appeared within twenty four hours of occlusion, and they lasted for varying periods—from a few minutes to several hours While the symptoms were usually more or less continuous a pain free period sometimes supervened before the onset of the syn drome of acute occlusion The anginal pain experienced by these patients was not influenced by nitroglycerin, as it is in typical angina pectoris, and this has been noted by others

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But if autopsy reveals an occluding thrombus, an entirely different opinion should be given and it is based on the fact that most coronary thrombi are produced slowly[30,31,44,49] A careful serial section through the occluding mass should have been made and it will probably reveal that its oldest portion was initiated hours or days before the workman collapsed and died In these circumstances, the opinion should be that the relationship between unusual exertion and sudden death was probably coincidental that the terminal thrombotic occlusion would have been just as apt to occur while the workman was at rest or asleep Indeed, in the presence of a gradually enlarging coronary thrombus one suspects that the reduction in coronary blood flow which occurs with rest is conducive to the final occlusion If true it explains why so many cases of coronary thrombosis die in their sleep[41]

From the foregoing it is evident that the demonstration of a relationship between stress and fatal coronary artery disease demands an expert and searching pathological examination it calls for more technical work than can be supplied by most clinical laboratories With even a cursory type of examination, however, one might argue that the demonstration of a coronary thrombus of any age implies that some part of it was initiated hours or days before the onset of cardiac pain and that the activities of the individual just prior to the onset of pain were probably coincidental

When no autopsy is performed or if the workman survives a bout of cardiac infarction which began at a time of stress one's opinion can only be based on probabilities Approximately two thirds of all myocardial infarcts[21] and one half of those in men under the age of thirty five[51] are due to coronary thrombi Thus if a workman develops a non lethal infarct in the course of his work there is at least an even chance that a coronary thrombus was at fault, and that this thrombus was probably initiated hours or days previously

It must not be inferred from the above that coronary thrombi cannot be initiated by stress through the mechanism of capillary rupture and intimal hemorrhage This hypothesis still holds, but the time relationships will be quite different from those in the case under discussion The unusual physical exertion experienced by the workman in question could have produced a small intimal hemorrhage and this in turn might eventually have initiated a thrombus However the thrombus would not have been apparent clinically for hours or days after the period of excessive exertion and during the interval other types of stress not related to work might have been the cause of the primary capillary rupture In my experience it is extremely difficult to establish a relationship between a particular bout of stress and a particular intimal hemorrhage of ordinary size even with the most searching type of pathological examination If the age of the hemorrhage agrees with the interval of time between the stress and death one can state that there may be relationship but this should be qualified by the admission that any other type of stress occurring about the same time might have been equally at fault

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Chapter

8

THE PROBLEM OF CARCINOMA IN SITU WITH REFERENCE TO THE HUMAN CERVIX UTERI

By Leland D Stoddard, M D

Introduction The Early Detection of Neoplasms and Their Origin in Fields of Abnormal Surface Epithelium

The problems of neoplastic disease today occupy an increasing share of physicians' attention because in the United States tumors account for about 1 in 7 deaths (1947), as contrasted with only 1 in 25 in 1900[1] That neoplasms now follow only cardiovascular diseases as a cause of death is in part a reflection of the decreased death rate from infectious diseases and of the increased proportion of our population forty years of age or older the age group in which neoplasms most frequently are found As long as a prosperous economy permits hygienic living conditions and wide application of scientific research, epidemiologic and chemotherapeutic successes over infectious diseases will enable more individuals to reach this older age group General concern about this growing problem is evident in the large sums spent from public and private funds for research and for professional and lay education in neoplasia Detection of tumors has become a problem in public health

The current cancer campaigns quite correctly stress that treatment will more likely be successful if a tumor is apprehended in an early and localized stage of its development This is especially true of the malignant epithelial neoplasms the carcinomas and will continue to be true as long as successful treatment depends on either surgical removal of all malignant cells or their destruction by an agent such as roentgen rays Although the growth rate of certain tumors has been partially controlled chemically notably prostatic carcinoma with estrogens it seems unlikely that fully developed carcinomas will be halted by such means in the predictable future Direct attack on etiologic agents is not promising because of the peculiar relationship of neoplasms to known carcinogenic stimuli both experimental and clinical Tumors may arise from fields of cells which have been exposed to a carcinogen in the distant past and in which the agent may be stored for years or may cease to be demonstrable For example arsenical keratosis and squamous carcinoma notoriously follow the administration of some arsenical compound years before Or in the case of mouse skin carcinoma induced by repeated painting of methylcholanthrene the neoplasm once established no longer requires painting for continued proliferation and repeated transplantation Such reactions stand in obvious contrast with say pneumococcal pneumonia an exudative inflammatory reaction which proceeds in the presence of the organisms and terminates when they have been destroyed In addition the wide range of

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The Field Concept of the Origin of Neoplasms —The development of carcinoma in situ and its relationship to invasive carcinoma is best understood by studying the histogenesis of different kinds of epithelial neoplasms Many observations point to their beginning as a field of abnormal epithelium The gross appearance of the field varies and often its limits can be determined only by microscopic examination The size of the field may be small or large The histologic evidence of abnormal growth within the field ranges from unobstrusive, hyperplastic thickening of the epithelium to flagrant cytologic atypicality that is usually considered characteristic of malignant neoplasms Sometimes epithelial atrophy is the most important abnormality It is not possible to specify the morphologic details of such fields apart from a discussion of individual kinds of tumors because each kind exhibits peculiar features The common feature in the histogenesis of most carcinomas arising from epithelial surfaces is restriction of the proliferation to the natural epithelial surface as long as the reaction is in the stage of hyperplastic transformation of the epithelium When assessed by morphological techniques the transitions from hyperplasia to neoplasia are gradual and insidious Later within this field of abnormal, hyperplastic epithelium invasion of underlying stroma may occur in one or many foci Even then however it seems possible that the surface field may continue to expand by the progressive incorporation of transformed contiguous epithelium Once invasive buds and pegs have penetrated the stroma the tumor is prepared to enlarge by intrinsic growth and to continue invading surrounding tissues If this stage of activity is attained the neoplasm is entitled by classical pathological criteria to the designation "carcinoma for as Nicholson[86] has pointed out ' The character which can fairly be said to distinguish all tumours from somatic tissues and organs is want of organismic control —surely intended by him as a description not an explanation The gross configuration of a neoplasm is of course variable but in time there often is a mass of new tissue formed by coalescence of downgrowths and by exuberant projections from the surface—in short a perceptible tumor or swelling Further extension of the tumor through lymphatic and vascular channels is possible after the tissue spaces of the connective tissue stroma have been invaded The abundance of metastases is undoubtedly a complex function of the extent of lymphatic and vascular invasion the anatomical configuration of lymphatic connections and vascular drainage and the ability of carcinoma cells to survive in the blood stream and in distant tissues and organs

The Multicentric Origin of Carcinomas in a Field of Epithelium Treated Experimentally With a Carcinogen —It is instructive to follow the sequence in the development of squamous carcinoma in an area of mouse skin treated experimentally with a carcinogen such as benzpyrene Since the dosage and the genetic composition of the animals can be controlled in the experiment the scheduled sacrifice of animals permits observations at progressive stages of the disease According to Glucksmann[44] a single application of benzpyrene produces epilation hyperplasia and squamous differentiation of the epidermis which is normally only two cells thick in the mouse Cellular degeneration and pre ulceration occur during the first twenty four hours and are followed by hyperplasia resulting from increased mitosis in the superficial epidermis Repeated weekly application of the carcinogen maintains the hyperplasia and after forty-eight to sixty days papillomas appear in the area Some of these papillomas are cystic and invaginated others are elevated Microscopically they are made up of partly keratin

stimuli—chemical, hormonal, irradiative, viral—known, or reasonably suspected, to be capable of eliciting a neoplastic proliferation further complicates clinical control. It seems reasonable, therefore, that extirpation of incipient and early tumors will continue to offer greatest hope in the treatment of human neoplasms.

Insistent demands that carcinomas be recognized, perhaps anticipated, in their early stages have focused attention on the lesion called carcinoma in situ. For more than forty years the lesion in the uterine cervix has been recognized and interpretation of it has been debated for more than twenty-five years, especially in this country and in Germany. The lesion is identified by microscopy and can be defined as an epithelial proliferation confined to surfaces but exhibiting to a greater or lesser degree the cytologic characters of obviously invasive carcinoma. The variety of designations given by authorities to this biological state of activity suggest various interpretations of its significance. In addition to carcinoma in situ, the following may be mentioned: non-invasive carcinomatoid change,[73] increasingly atypical cervical epithelium,[131] non-invasive intraepithelial carcinoma,[66] or intraepithelial carcinoma, non-invasive carcinoma,[35] pre-invasive carcinoma,[99] incipient carcinoma,[20] early carcinoma.[114] In 1910 Rubin[10] presented in the American literature an accurate description and illustration of the lesion. Further, he proposed that cytologic atypism is an adequate criterion of malignancy even in the absence of invasion of underlying stroma. Rubin's early study belongs with a small group of classic observations published in the German literature just before 1910. Almost twenty years later Schiller[110, 111] concluded from his extensive investigations that he was able to recognize the lesion by histologic examination and he repeatedly insisted that such superficial proliferations be labeled carcinoma, thus eliminating their ambiguous status. Meyer[80] supported the same viewpoint. However, others have contended that a carcinoma is recognized by its behavior, namely, invasion and destruction of neighboring tissues and distant metastasis via lymphatic and vascular channels. Meyer recounts in several places his debates with distinguished German pathological anatomists who took that position. Those who are interested in the personal side of this argument will find interesting comments in Dr. Meyer's autobiographical recollections.[81]

It is unfortunate that such "it is malignant" — "it is not malignant" arguments often depict neoplasia as a predetermined condition and thus hinder an understanding of the dynamic developmental aspects of the process. It is understandable that those who must treat a patient would prefer to know whether or not a carcinoma is present, but an invasive carcinoma does not suddenly arise today in an area that was entirely normal yesterday. Since the developmental stages antecedent to invasion have become recognized, well-intended demands for early extirpation of neoplasms have been extended to include them too. But the recognition of these stages, such as carcinoma in situ or leukoplakia or hyperplasia with atypicality, raises problems in determining for each kind of lesion the frequency of progression and invasion. Discriminating morphological, chemical or experimental techniques for predicting the outcome of each individual case would be highly desirable, but they are yet an aspiration for the future. These problems are not solved—they are ignored—by simply labeling carcinoma in situ as "carcinoma" and then assuming that it should be treated like carcinoma.

It will be worthwhile to sketch the morphogenesis of certain epithelial neoplasms, experimental and human, before considering the origin of squamous carcinoma of the cervix in detail.

Willis's[13] studies clearly demonstrate the field origin of epidermal squamous carcinomas and do not support speculations that these tumors arise from a single cell or small group of cells. His observations can readily be confirmed in any pathological laboratory receiving surgically excised dermal tumors. If hyperplastic keratotic lesions such as senile keratoses or atrophic lesions like those of discoid lupus erythematosis are also examined, stages of carcinogenesis even earlier than the ones depicted by Willis can be found. As he has illustrated, small carcinomas insensibly merge with hyperplastic margins of epithelium which sends deep and irregular pegs into the corium. The central portions may present a variety of gross forms—invaginated craters with rolled margins, elevated papillomas, hyperkeratotic verrucae or horns, but all are characterized by exaggerated hyperplasia and deeper, blunter pegs extending into an inflamed corium. Although the central tumor usually is well differentiated, it exhibits the greatest degree of cytologic atypism and dyskeratosis. The bulk of the tumor increases by intrinsic growth of downward projections, marginal undermining, upward projection, and probably by conversion of the lateral hyperplastic field to neoplastic epithelium. Invasive foci in the field are usually multiple and often penetrate the stroma in areas of degenerated elastica. The determination of just what constitutes invasion can, no doubt, be debated, but I have found a useful criterion that is applicable to quite small growths as well as to large ones. Within the hyperplastic field appear buds of well differentiated polyhedral cells which push into the stroma and are not surrounded by a layer of basal cells. The juxtaposition of larger polyhedral cells and stroma would seem to be acceptable evidence of abnormal relationship of epithelium to connective tissue, although there are exceptions to the rule. For example, regenerating epithelial cells at the very margin of an ulcer or extending along the floor of an epidermal dermal bulla may not have the characteristics of basal cells.

Squamous cell carcinomas of the lip develop in the same way. These tumors imperceptibly fade into a surrounding hyperplastic zone from which there is no sharp demarcation. In a case described by Willis[133] a field of origin including both vermilion border and buccal epithelium was clearly indicated because the invasive pegs from the epidermal side were acanthomas better differentiated than those from the buccal portion.

Willis[134] has also illustrated widely separated invasive foci in large carcinomatous surface fields arising in chronic ulcers and in burn scars. In such cases the lesions differ from the smaller tumors only in the extent of the hyperplastic field and in the fact that the multiple invasive carcinomas may be widely separated. The duration of the hyperplastic stage is often very long, the incidence of invasive carcinomas developing in such fields is low, and their progress is slow. Certainly hyperplasias of this kind seldom progress to neoplasia, but whether this progression, when it occurs, depends on some additional stimulus is not known. One naturally asks whether there are surface hyperplasias essentially benign but susceptible to neoplastic conversion, contrasted with others destined for a malignant career from the outset.

In a consideration of the multicentric origin of neoplasms, multiple foci of invasion within a continuous field should be distinguished from multiple discrete fields. In the cases just mentioned, multiple invasive foci can be demonstrated within a circumscribed, uninterrupted field of hyperplastic epithelium. The field is often identified by an elevated hyperplastic lesion with gently fading acanth

ized excrescences and downward projections. The hyperplasia involves not only the superficial epidermis but also the hair follicles which are transformed into elongated interpapillary processes. Lateral extension of the papillomas occurs by progressive marginal change. Some papillomas regress and others persist. As early as the seventieth day, malignant foci appear both in gross papillomas and in intervening areas. These foci are marked by an increase in mitosis, an increase in the proportion of resting, or undifferentiated basal cells (anaplasia), an increase in volume of cytoplasm, nucleus and nucleolus, and by invasion of underlying stroma. In Glucksmann's experimental tumors as in many human neoplasms the underlying stroma was altered by inflammatory reaction and dissolution of basement membranes, but with the use of a suitable solvent for the hydrocarbon inflammation is not a necessary accompaniment of experimental carcinogenesis.[93] Invasive foci may coalesce in time. It is worth emphasizing that the reaction in the treated area is generalized although variation in its intensity produces focal tumors, that multiple invasive foci often arise in an animal and that Glucksmann found no morphological evidence that the changes resulted from rapid proliferation of a single cell.

This model for the histogenesis of squamous carcinoma experimentally produced in mouse skin could be taken with some variations from the observations of Orr,[93] Cowdry and Paletta,[15] Cramer and Stowell[16] and others. Although we have depicted the process as one of steady progression without demonstrable boundary between hyperplasia and neoplasia, some investigators consider that the events of carcinogenesis are discontinuous. Cramer and Stowell[16] pointed out that in a wide field of hyperplasia induced by repeated application of methylcholanthrene stationary hyperplasia was the rule, whereas progression to carcinoma like regression was less frequent. Although the carcinomas they produced were restricted in area, the tumors seem best understood as proliferations developing over an area of epithelium rather than from a single cell. They too reported the development of independent neoplastic foci in a given animal.

Presentation of this example from experimental oncology to illustrate the field origin of carcinoma does not imply that the particular carcinogen is important in the causation of cervical carcinoma in the human being. Nor does it imply that the cytochemical alterations demonstrated in the experimental condition are known to hold equally for cervical carcinoma. In fact, it may be doubted that the fundamental process in such acute experiments is analogous with the development of a surface field of highly abnormal epithelium that remains non-invasive for a long time. In similar experiments with methylcholanthrene Murphy[84] has produced transplantable squamous carcinoma of the cervix and vagina of the mouse. But in only a single instance was a field of atypical surface epithelium resembling human in situ carcinoma produced. Generally the early focal lesions were already invasive.

Examples of the Field Origin of Certain Human Carcinomas.—Does similar conversion of a field of surface epithelium to a pre-invasive hyperplastic neoplastic state occur in the histogenesis of human carcinomas? This is a critical question because it is often asserted that a tumor has a unitemporal unicentric origin from a single cell or a few related cells and that it enlarges by division of its intrinsic cells. We shall confine our considerations largely to certain illustrative carcinomas originating in surface epithelia.

vasive foci within the field This observation is also true of the cervix But in contrast with vulval and other well keratinized epithelia, leukokeratoses of the portio epithelium seldom are fields from which carcinoma develops This fact that cervical leukoplakia is usually benign has been repeatedly demonstrated

It is not our purpose to review here all the evidence supporting the concept that in the human being carcinogenesis is frequently a two stage process involving hyperplastic neoplastic transformation of a field of normal epithelium followed by increasingly intrinsic growth of the tumor, invasion and dissemination No mention has been made of the histogenesis of adenocarcinoma of the colon and of the endometrium Both neoplasms provide striking examples of this mode of origin Adenocarcinoma of the colon often arises in a polyp covered with atypical epithelium and endometrial carcinoma in a field of hyperplastic endometrium In the illuminating studies made possible by full histologic sections of breasts Cheatle and Cutler[1] found that areas of intraductal epithelium undergo neoplastic hyperplasia in situ and that along these surfaces invasion may occur at one or more sites There is not space to cite examples from parenchymatous organs such as the liver where primary hepatic cell carcinoma is often preceded by prolonged regeneration in cirrhosis Willis[134] has stressed the development of many neoplasms from fields of transformed epithelium and has critically discussed shortcomings of the concept of a strictly unifocal origin One may conveniently refer to his bibliography for many of the observations supporting the concept of a field of origin they are not new One can only suppose that the inclination to ignore or to explain away such observations stems from an attitude of censure toward a fully developed malignant neoplasm The destructive invasion of an advanced carcinoma is so different from the results of normal cellular activities that the havoc is conveniently blamed on a new race of cells deriving from a sudden mutant This idea is not supported by thorough histologic investigation of the developmental phases of many carcinomas This is the case with squamous cell carcinoma of the uterine cervix

The Development of the Concept of the Field Origin of Cervical Carcinoma —During the past half century evidence accumulated from a number of independent investigations strongly suggests that invasive squamous carcinomas of the cervix uteri arise in surface fields of atypical hyperplastic epithelium which have been present for variable periods of time Histologic observations on excised material have quite often been advantageously correlated with gross observations in the course of repeated clinical examinations For these contributions to an understanding of carcinogenesis in the human cervix we are largely indebted to gynecologists and gynecological pathologists interested in the early diagnosis of uterine carcinoma We have already noted that Rubin recognized the field origin of cervical carcinoma and he discovered lymphatic invasion from the surface field[105] In 1908 two years earlier Schauenstein[108] comparing 4 cases depicted a stage of cervical carcinoma in which a coat of atypical squamous epithelium was spread over the surface and in glands and a later stage in which there was deeper invasion He thought that there were only quantitative differences in these stages of one and the same malignant neoplastic growth process A year later Pronai[88] presented 3 similar cases In summarizing his study on the histogenesis and growth of uterine carcinoma he plainly stated that it was hardly necessary for carcinoma to begin always from embryonal rests (Cohnheim) or displaced epithelial cells (Ribbert), but rather in some cases the surface epi

otic, hyperkeratotic margins not clearly demarcated from the central acanthoma But in conditions such as xeroderma pigmentosum arsenical keratosis and recurrent epitheliomatosis of the exposed surface of fair complexioned persons, multiple discrete tumors occur in an extensive area of epithelium which seems to be potentially prepared for neoplastic transformation Although this is a proper distinction microscopic study sometimes discloses that apparently discrete lesions actually are foci of exaggerated activity in a general field of morphologically atypical hyperplastic epithelium This is often true of vulval leukoplakia and early carcinoma The same might be said, too, of *x* ray dermatitis in which the recurrent development of gross tumors in a field of atypical epithelium is a matter of common experience All these examples are, however less akin to cervical in situ carcinoma than is another category of epidermal field changes

This second general group of dermal neoplasms illustrates a field of origin more comparable to cervical carcinoma in situ because cytologic atypism rather than acanthosis and hyperkeratosis is the paramount characteristic The group includes Bowen s disease Paget s disease of the nipple and erythroplasia of Queyrat It is the striking cytologic atypicalities within the field which set these lesions apart *Whereas the early carcinomas and keratoses previously described present* as well differentiated acanthomas exaggerating the normal pattern of the epidermis, the latter dyskeratoses are marked by a full thickness of densely packed, highly abnormal cells exhibiting nuclear hyperchromatism, variability and atypism and premature keratinization of individual cells the ' grains " There may or may not be evidence of superficial parakeratosis and hyperkeratosis Although certain clinical findings and certain histological details such as the clear cells of Paget s disease, may justify separation of the group into entities the essential point in all is that a field of surface epithelium is converted to an area of highly variable epithelium exhibiting the cellular atypism of carcinoma This epithelium confined by natural boundaries may persist for years as in Bowen s disease and then be followed by invasive carcinoma in the area As far as the principle of histogenesis is concerned it is not important that the field in Paget s disease includes the large mammary ducts as well as the nipple, and that invasive carcinoma arising in the field resembles other carcinomas of the breast and metastasizes to axillary lymph nodes In this group too the field may be continuous or discontinuous

The occurrence of multiple foci of invasion within a field of prepared epithelium is common to all the lesions discussed thus far but the histologic characteristics of the field have prompted a division into an acanthotic group and a less well differentiated or anaplastic group with more obvious cytologic atypism But this dichotomy is not absolute For example the atypicality found in some senile keratoses causes them to resemble Bowen s disease Vulval carcinomas particularly exhibit great variation in morphologic composition of a single wide field Portions of the field may be parakeratotic and keratotic leukoplakias in which verrucous excrescences develop and other areas less conspicuous grossly may resemble Bowen s dyskeratosis If many blocks are routinely prepared from such lesions there is often no difficulty in demonstrating invasive foci throughout the field irrespective of the basic cytologic pattern or degree of differentiation Exactly the same situation can be found in carcinoma of the larynx and of the tongue It is clear then that the degree of differentiation of the surface field bears no determining relationship to the appearance or non appearance of in

Although progressive degrees of cytologic atypism leading to fully developed in situ carcinoma will be discussed later it is convenient to restrict the present description to lesions in which the cellular aberrations are like those seen in obviously invasive carcinoma Indeed it is often said that the cytologic alterations in in situ carcinoma and in invasive carcinoma are identical but evidence will be presented to show that this is not always true It is appropriate to refer here to the report of Rubin His description and drawings need not be changed in any material way to illustrate the essential cytologic abnormalities in a surface field of carcinoma in situ In describing his 3 cases he agreed with a few earlier observers that the atypical epithelium presented the following ' outspoken abnormalities

'1 An indistinct uncertain definition of cell outline particularly in the deeper layers (germinal proliferating)

2 The presence of irregular, large intensely stained nuclei occasionally grouped in clumps

3 No definite stratification only partial parallelism of the basal cells (more often they are seen to be irregularly disposed or at a slant toward the tunica propria)

4 The marked nuclear granulation This sign deserves special attention because according to Schottlaender it is very frequently seen in carcinoma which has not yet undergone cornification '[105]

Fundamentally the lesion is recognized by the nuclear abnormalities of the epithelial cells Densely granular chromatin, in contrast with normal finely powdered chromatin fills enlarged round or oval nuclei A considerable degree of nuclear uniformity is typical but some lesions present obvious variability in nuclear size and shape Mitoses usually are found in abnormal numbers and significantly may appear well above the basal row on occasion they are abundant but not necessarily in all parts of the field Nucleoli are prominent

The crowded nuclear population attracts attention to this abnormal surface layer when it is viewed with low magnification It is usually about the same thickness as the normal portio epithelium Often undifferentiated cells containing little cytoplasm and hyperchromatic nuclei slanting toward the base are uniformly packed throughout the entire thickness Sometimes such an epithelial coat is surmounted by a superficial parakeratotic layer of cells with conspicuous pyknotic nuclei and brightly eosinophilic cytoplasm (Fig 45D) This differentiation is abrupt so that the elongated cell bodies in the parakeratotic zone running at right angles to the long axes of the undifferentiated cells below divide the epithelium into two distinct zones In other cases there may be three zones caused by an intervening middle layer of differentiated polyhedral or prickle cells containing more cytoplasm than the cells in the undifferentiated basal layer beneath (Fig 45F) It can be seen therefore that the abnormal epithelium cannot always be recognized by failure of differentiation for the degree of differentiation is inconstant Two further variations in the field are encountered occasionally At times only the lower half of the epithelium is composed of abnormal cells and sometimes even though it is abnormal from base to surface the epithelial coat is thin It is doubtful that these are consequential variants because they can accompany the conventional lesion

Schiller[119] studied in detail the intracellular vacuoles that stand out at times (Fig 45E) He took them to be both paranuclear and intranuclear and found

thelium transformed itself directly into carcinomatous epithelium In 1922 Martzloff[69] presented an excellently documented report of a case in which only a small focus of carcinomatous invasion was found in an extensive surface field These observations and the inferences to be gathered from the voluminous studies of Cullen[17] and of Schottlaender and Kermauner[116] seem to have been lost sight of For example, Wespi[131] says of Hinselmann s studies in the 1920 s

" when [he] constructed his colposcope for the investigation of the early forms of cervical cancer, he started with the idea which at that time was generally accepted namely that the early form is a kind of miniature of the advanced cancer, *that is, a small carcinoma node He himself*, was quite surprised when he found an entirely different mode of development Hinselmann discovered that the expected little cancer nodes appear very seldom as early stages of carcinoma but he found increasingly atypical [surface] epithelium comparatively frequent In other words the first phase of cancer growth is *only cytologic change in the* cervical epithelium, an epithelial change expanding on the surface "

Schiller's detailed studies of cervical carcinoma seem to have firmly established the idea of a two stage development of the neoplasm His lectures in this country served to call attention to the independent observations of Pemberton and Smith[97] which supported this same concept Meyer too, endorsed surface carcinoma as an early pre invasive stage of genuine carcinoma and Hinselmann[5] placed himself beside Schiller and Meyer From what has already been said it is expected *that Martzloff[70–73] recognized atypical surface epithelium as a possible stage in* the development of invasive carcinoma but taking a more restrained view, he did not label it out right carcinoma Other contributions have been made to a number of which there will be occasion to refer

In the discussion to follow we shall be concerned largely with the identification and histogenesis of these in situ fields of surface carcinoma and the evidence which points to them as a stage in the development of invasive carcinoma Even though special conditions and variations that apply to the human cervix must be considered it has already been indicated that the same general principles of carcinogenesis apply to other anatomical sites especially epithelial surfaces We shall note that microscopic invasive lesions occasionally have been found in cervical epithelium which does not have the cytologic characteristics of carcinoma in situ As the discussion develops we shall inquire how far available evidence supports the idea that in a field of atypical epithelium progression to an invasive destructive disseminated growth is inevitable the problem of reversibility and regression of the lesion in question will also be dealt with In proper place certain experimental data which contribute to an understanding of cervical in situ carcinoma will be referred to Finally we shall consider the relationship between the pathogenesis and the detection and treatment of carcinoma in situ of the cervix

THE CHARACTERISTICS AND THE HISTOGENESIS OF CARCINOMA IN SITU OF THE CERVIX UTERI

The Histologic Characteristics of Fully Developed Carcinoma in Situ of the Cervix – Since there are no constant and few reliable gross characteristics of this lesion, we can pass at once to its identifying microscopic characteristics

parallel strands of flattened polygonal cells with little cytoplasm and pyknotic nuclei. The canal is lined by a layer of simple columnar mucous epithelium that extends deep into the stroma as branching endocervical glands. Almost always at least a few small undifferentiated "reserve cells"[6] lie beneath this cylindrical epithelium. Because of their position they are also called "subcylindrical basal cells." The two kinds of epithelium join near the external os. Intermingling growth of squamous epithelium and columnar epithelium near the os establishes a *junction or transformation zone* in which both types of epithelium can be seen. In some cases the endocervical epithelium extends far onto the face of the cervix, especially on the anterior and the posterior lip. In other cases squamous epithelium extends for some distance up the canal. This admixture of two kinds of epithelium was described by Robert Meyer[76,77] in terms of a struggle whereby first squamous and then mucous epithelium gains the upper hand. His observations and also his interwoven interpretations are widely accepted. The observations can be readily confirmed. In the majority of cervices histologic study reveals some endocervical glands and perhaps lining mucosa on the face of the cervix beyond the external os, a state I shall refer to as an *endocervical ectopia*. One seldom comes upon a pure ectopia, for the squamous epithelium usually covers most of the surface, forms lips for the mouths of glands, or even extends into them displacing their columnar epithelium. Within the stroma there is invariably edema and a chronic inflammatory exudate of lymphocytes and other mononuclear cells. Abundant capillaries loop to the surface so that a varying degree of hyperemia is visible beneath the squamous epithelium, which is often thin and appears to be regenerating (See Fig. 46D). If inflammation and edema are severe the thin epithelium is easily macerated and abraided. This causes acute ulcers or *erosions*. The alternating glandular pits and vascular loops produce a granular surface, and growth of the portio epithelium into the mouths of glands and over capillary loops results in a wavy stratum basalis with undulating epithelial pegs extending into the inflamed stroma. The glandular orifices appear as pinpoint gray dots and when they are obstructed by the regenerating squamous epithelium the glands dilate to form the familiar Nabothian cysts filled with mucus and often polymorphonuclear leukocytes. Progressive dilatation causes atrophy of the epithelial lining, acute inflammatory ulceration and intensification of surrounding interstitial inflammation. Regression of the ectopia is marked by disappearance of glands, subsidence of inflammation, decrease of vascularity and establishment of a full thickness layer of squamous epithelium. In an otherwise normal cervix a few scattered Nabothian cysts and some irregular pegs of portio epithelium are generally considered marks of a previous ectopia. It must be understood that the lesion may extend beyond the visible ectopia, for the proliferation of squamous epithelium can extend the transformation zone into the canal resulting in *epidermization* of the endocervix*. It would perhaps be more nearly correct to refer to the transformation zone as a zone of mixed epithelium because neither type of epithelium can be seen converting into the other; the two types exist together in the same area, usually squamous on the surface and columnar in glands.

The transformation zone is further complicated by the presence of the small indifferent "subcylindrical basal cells" or "reserve cells" of the mucous epithelium

* There are many synonyms including *epidermalization*, *epidermidalization*, *epidermoidalization*, *epidermalization*. Perhaps *squamous epithelization*, a term that avoids reference to epidermis, would be preferable.

that they contained one or more brightly staining hyaline bodies. They are often found in the normal squamous epithelium at the margins of a carcinoma in situ. Such vacuoles are seen along with widened intercellular bridges, the two findings probably indicate intra and intercellular accumulation of fluid, an accompaniment of edema. The same changes occur in inflammation of the cervix.

Less emphasis is usually placed on cytoplasmic alterations but several are important. In undifferentiated lesions cytoplasm is scanty in the cells of all layers and is usually basophilic or amphophilic. Cytoplasmic basophilia is often found in the presence of enlarged nucleoli likely indicating nucleolus associated nucleic acid synthesis. But even when the lesion is better differentiated the polyhedral or prickle cells lack the clear cytoplasm that surrounds the crinkled nuclei of the cells composing the normal upper malpighian layer. McManus and Findley[74] have demonstrated with histochemical techniques that these cells lack the intracytoplasmic, water insoluble glycogen of normal portio epithelium. Therefore, they do not stain brown with Schiller's iodine test. Peculiar, intensely eosinophilic intracytoplasmic granules are uncommonly found.

Two manifestations of abnormal differentiation or dyskeratosis, should be noted even though they are not always present. Individual cells anywhere above the basal layer may abortively keratinize, such cells exhibit the small pyknotic nuclei and shrunken coagulated acidophilic cytoplasm of the "grains" of Darier. A more ominous sign is the presence of rounded, intraepithelial pearls or pseudo pearls below the surface. These complexes representing clusters of prematurely differentiating cells may give rise to genuinely invasive, microscopic buds as we shall emphasize later.

It is apparent that no single histologic formula can include all the combinations of cellular atypicalities that are encountered. Only some of the variations are illustrated in Figures 45, 46 and 51. Nuclear atypism is the constant feature. All other characteristics—frequent mitoses, aniso- and poikilocytosis, failure of differentiation, intracellular vacuolization and dyskeratosis—are variable and inconstant.

The Problem of the Histogenesis of the Field of Cervical Carcinoma in Situ.—Unanimity of opinion concerning the histogenesis of carcinoma in situ of the cervix does not prevail. Theoretical considerations disregarded, this problem is involved in a discussion of practical matters regarding the *position and distribution* of the lesion. Squamous cell carcinoma of the cervix is to be expected on the face of the cervix covered normally by squamous epithelium, but the neoplasm also arises in the cervical canal; this also is true of the surface fields *of carcinoma in situ from which the invasive tumors originate.*

The Transformation Zone in the Cervix, Endocervical Ectopia, Squamous Metaplasia of the Endocervix.—Discussion of the anatomical distribution of in situ carcinoma and its histogenesis must be based on the *epithelial transformations* regularly progressing in almost every adult cervix. The exposed intravaginal face of the cervix, the portio vaginalis, is normally covered to the external os by a non keratinized squamous epithelium as shown in Figure 45A. The basal layer is composed of a single basal row of columnar cells and one or two layers of small polyhedral cells forming an irregular mosaic. Because these cells quickly acquire a large quantity of glycogen, most of the regularly stratified malpighian layer is made up of larger polyhedral cells with abundant clear cytoplasm and a small nucleus. The desquamating surface layer consists of a few

"The benign epithelization represents the healing of a cervical erosion. An inflammatory infiltration most often spread from the cervical mucous membrane, comes to lie beneath the squamous epithelium of the portio. The destructive effects of this progress toward the surface until all layers are destroyed and the erosion is produced. The mucus producing epithelium of the cervix or its glands grows over the defect and this represents the first stage of the erosion healing. As the inflammation subsides the squamous epithelium advances from the margin of the erosion. It grows by a proliferation of its basal cell layer between the connective tissue and the mucous epithelium and may similarly extend into the glands. The mucous epithelium is lifted off and dies. Finally both in the glands and on the surface basal cells differentiate into layers, lose their unripe character, and may fill up the glands as well as cover the surface. This process of ripening may proceed slowly. The filling of the glands may take considerable time and mucus producing epithelium may persist during this process. To understand the epidermization in the higher level of the cervical canal and also of polyps arising from this area it must be clearly understood that in some areas basal cells exist beneath the mucous epithelium. These cells have remained in this area since the early developmental stages as fetus and child at which time the entire cervical canal was covered with the squamous epithelium of the vagina. This was pointed out by the writer thirty years ago and confirmed by later work on the development of the vagina."[79] In another place[78] he reaffirmed his previous conclusions and stated that the see saw battle of healing and progression in adult inflammatory erosions also results in sequestration of islands of basal cells, thus he proposed two conditions in which islands of subcylindrical basal cells or reserve cells become isolated in the endocervix. In Meyer's view then the subcylindrical basal cells represent either direct undermining growth of basal cells of the squamous epithelium or else islands of these same cells sequestered during fetal, childhood or adult life.

Carmichael and Jeaffreson[8, 9] studied the subcylindrical basal cells and their proliferation apart from the problems of ectopia or erosion and marshalled most of the evidence against Meyer's hypotheses. In tracing the process of squamous metaplasia they noted that this abnormal epithelium covering the endocervix showed great variation even in the same cervix. It ranged from epithelium obviously related to the columnar epithelium to squamous epithelium almost indistinguishable from that of the portio vaginalis, including a store of cytoplasmic glycogen. They held that a clear cut demarcation line between metaplastic and portio epithelium and the demonstration of mucus in the metaplastic epithelium with the muci carmine technique did not support Meyer's views. These investigators even accepted the possibility of direct metaplasia of columnar to squamous epithelium but Meyer's well known objections to this idea are generally acknowledged. To quote from their writings:

"In our view then the patches of atypical multilayered epithelium frequently found in the endocervical mucosa are formed from the original endocervical lining, the squamous epithelium playing no part in their development. They may be derived from surface columnar cells alone but very frequently pre-existing basal cells play a predominant part in their formation. Since the epithelium produced in this way may closely resemble squamous epithelium the pathological process may be looked upon as an incomplete squamous metaplasia."[9] Referring to the lower canal however they say: "When typical squamous epithelium is

They are comparable with similar cells in respiratory epithelium, but in the cervix their presence is less constant and their development more irregular. The nuclei are small, round and quite evenly filled with darkly *staining chromatin in which* a nucleolus is not visible (Fig 46B). Slightly larger ovoid, somewhat vesicular nuclei are also found. Usually little cytoplasm is seen. Sometimes the reserve cells merge imperceptibly with the stroma, but usually they rest on a layer or two of collagen fibers. In the endocervix a hyaline basement membrane like that in the bronchus seldom develops. These cells are important because they have a *latent potential to undergo hyperplasia and to differentiate to squamous epithelium* as beautifully illustrated by Carmichael and Jeaffreson.[8, 9] Different degrees of squamous metaplasia are seen beneath the columnar epithelium of the endocervical gland illustrated in Figure 46A: below, the reserve cells remain undifferentiated, but above they have differentiated to polyhedral cells. In early stages like this the columnar epithelium is elevated. Then it atrophies and finally disappears so that by *indirect metaplasia* the endocervical mucosa on the surface and along glandular linings may become squamous. This substitution of squamous for columnar epithelium is most frequently seen about the external os but may occur high in the canal, often in islands, and on the face of the cervix in ectopias. When fully developed metaplastic epithelium sharply abuts on the portio epithelium, *the two are distinctly delineated as illustrated by Howard, Erickson* and Stoddard.[6] The metaplastic epithelium can be distinguished from the portio epithelium by its basal row composed of round cells resembling the reserve cells rather than columnar cells, by a disorderly cellular dispersal rather than a regular stratification in the upper layers, and by deficient glycogen storage. Near the os, however, and on the face of the cervix it may differentiate so well that no cytological distinction or demarcation line sets it off from the squamous epithelium of the portio vaginalis. Such a length of squamous epithelium which merges imperceptibly with the portio epithelium on the face and with subcylindrical reserve cells in the canal might be interpreted, after Meyer,[76, 77] to arise from proliferation of squamous epithelial basal cells that undermine the columnar epithelium in a lava-like flow up the canal.

We thus see that the transformation zone may be a wide or a limited area centering on the external os and moving in either direction. Endocervical mucosa and glands may become established on the face of the cervix in ectopias, and it seems entirely likely that from the margins hyperplastic portio epithelium can regenerate over the surface of the lesion, continue along the glandular linings and even extend *into the endocervical canal. It is also generally agreed that the* mucous epithelium is frequently replaced through hyperplasia and squamous differentiation of the subcylindrical reserve cells. Both mechanisms may be responsible for *squamous epithelization of ectopias* and for *epidermization of the endocervix*, both surface and glands.

Meyer's explanation of the origin of ectopias and of the reserve cells is popular although not uniformly accepted. His thesis rests on two sets of studies,[76, 77] one in the adult and one in the fetus. He considered the endocervical ectopia in the adult as the first stage of healing of an inflammatory ulceration or erosion of the squamous epithelium and termed the ectopia a "pseudoerosion." As Meyer[81] insisted, his German publications must be consulted for a full exposition of his theory, but he himself *summarized* it in English.

The Development of Carcinoma in Situ in the Portio Vaginalis Epithelium —Carcinoma in situ arising on the face of the cervix usually develops within the portio epithelium of the transformation zone of an endocervical ectopia Younge, Hertig and Armstrong[133] found an erosion present in about 80 per cent of their 135 cases Wespi[131] also *comment on the frequency of ectopia* Meyer[80] insisted that the earliest lesions appear at the margin of the pseudoerosion never within it and that from the marginal zone the superficial carcinoma may spread along the surface in either direction Those who use the Schiller test therefore point out that a small non staining zone of surface carcinoma at the margin of an erosion area (ectopia and transformation zone) should not be *overlooked or mistaken for part of the non staining ' erosion itself* Although a genuine erosion stains most of the ' erosion area does not stain because it is covered with mucous epithelium or thin squamous epithelium The surface carcinoma does not stain because its squamous cells lack cytoplasmic glycogen

An endocervical ectopia or pseudoerosion is the only gross lesion frequently associated with in situ carcinoma of this kind Certainly not every ' eroded cervix harbors a carcinoma in situ in the transformation zone but unfortunately the one that does is seldom more suspect than any other inflamed endocervical ectopia For instance among the 135 cases of carcinoma in situ reported by Younge *et al* [133] over 100 lesions were grossly thought to be benign erosions and only 6 were suspicious because of easy bleeding after manipulation Sometimes there may be no gross abnormality to call attention to a latent carcinoma in situ

Even if the face of the cervix is obviously abnormal the field of the surface carcinoma does not necessarily occupy all of the transformation zone or necessarily confine itself only to the areas of most severe inflammation and erosion Foote and Stewart[31] investigated the anatomic distribution of carcinoma in situ and concluded that the extent of the field cannot be identified by the gross appearance of the cervix They mapped surface carcinomas from the study of histologic sections of serial sagittal blocks of entire cervices such a map could then be compared with a photograph of the gross specimen taken before it was cut In general the field does lie in the transformation zone and its margin Nevertheless the squamous epithelium well outside this area may be converted to carcinoma in situ which occasionally extends even to the surrounding vaginal epithelium Rarely the entire vagina, as well as the portio vaginalis of the cervix is involved Wespi[131] *has illustrated such a case and referred to similar cases in the literature* Since the portio epithelium in a transformation zone may grow into the cervical canal it is not surprising all or that a part of the field can be hidden beyond the external os

Histologically the surface coat is composed of abnormal epithelium exhibiting some combination of the cytologic atypicalities already described The surface carcinoma usually is well differentiated and characterized by prominent intercellular bridges which produce a mosaic pattern just above the basal row Some covering parakeratosis is common but there seldom is a heaped up or thickened keratinizing lesion *i e* leukoplakia Although most leukoplakic lesions of the cervix lack significant histocytologic atypism and are entirely benign an occasional carcinoma in situ will present the gross features of leukoplakia and thus resemble a few surface carcinomas reported by von Franque[3] [33] and by Hinsel

found in the canal it is usually continuous with the pre existing squamous epithelium and is always so situated that its origin by ingrowth cannot be excluded "[9] Much of the earlier work on the problem is referred to in a paper by Fluhmann,[29] who traced the process of "epidermidalization in much the same way and called attention to similar changes in the endometrium and in polyps attached high in the endocervical canal The more recent studies of Auerbach and Pund[3] and of Howard *et al*[6] are also in agreement with the conclusions of Carmichael and Jeaffreson It is surely true that from a study of many cervices observations can be assembled to favor each point of view, and certain cases can be interpreted in whichever way one chooses Often, however, a thin film of regenerating portio epithelium *proliferating over an eroded area of an ectopia quite plainly does not* undermine the adjacent columnar epithelium of underlying glands or surface but might readily replace the columnar epithelium if it were lost In my opinion, extensive subcylindrical epithelial hyperplasia bringing about squamous substitution or indirect metaplasia, is usually the result of autochthonous proliferation of the endocervical reserve cells

Little more than contributory causes can be suggested for endocervical ectopia and squamous metaplasia of the endocervix The two can hardly be considered etiologically unrelated because they so regularly appear together in the same transformation zone Meyer s theory that the ectopia in the adult is merely the healing stage of an inflammatory cervical erosion has already been discussed It is *extremely difficult to know the exact pathogenetic sequence of development* however Inflammation of some degree is quite regularly present in the cervix and even Meyer[81] stated that the pure first stage of an erosion is rarely encountered The inflammation might well follow the inevitable trauma to a mucous surface in a relatively exposed place The frequent appearance of ectopias during pregnancy suggests that endocrine factors may be involved in the extension of the mucous epithelium onto the face of the cervix Perhaps the excessive production of mucus by the columnar epithelium during pregnancy macerates the squamous epithelium to produce an erosion and subsequent inflammation followed by growth of the mucous epithelium into the area It has frequently been suggested that ectopic endocervical epithelium on the portio vaginalis results from lacerations or ectropions incident to delivery However these are hardly satisfactory explanations of the more or less symmetrical ectopia appearing regularly on the anterior and posterior lips and usually avoiding the lateral margins of the os where lacerations are commonest Because of the unsolved problem of the *etiology of the lesion I prefer Hinselmann s[58] non committal* designation "endo cervical ectopia which implies no particular cause In the case of the other condition under discussion squamous metaplasia several studies indicate that the incidence increases with age This suggested to Auerbach and Pund[3] that unopposed estrogenic stimulation might be *responsible for the reserve cell hyper* plasia These workers called attention to experimental support for this idea in the fact that administration of estrogens in animals produces the change Furthermore Carmichael and Jeaffreson[9] mention that progesterone mitigates the reaction in experimental animals In the human being this change *bears no* constant relationship to lacerations or ectopia and inflammation is so common that no significant correlation with it is possible

This proliferative and regenerative activity of the epithelium in the transformation zone provides the basis for the establishment of fields of cervical in situ carcinoma

the left in these illustrations) ending just beyond its limits Unusually severe inflammatory reaction sometimes obliterates the sharp connective tissue layer normally separating epithelium from stroma As we shall discuss later it may be extremely difficult in such cases to determine whether the stroma has actually been invaded Even if there is little inflammation, the pegs of the surface epithelium dip irregularly into the granulation tissue and the field may extend along the surface into endocervical glands present in the ectopia If there is much edema, the epithelium is lifted up by subepithelial bullae and subsequently dies leaving an erosion Because of inflammation and edema the carcinoma in situ may be so fragile and easily abraided that slight manipulation causes bleeding from the abundant vascular network below

Schiller[11] and Meyer[80] too, held that the cytologic atypicalities were always full blown entirely without transitions to normal epithelium and obvious to the experienced observer Schiller[112] called attention to the sharp line which sometimes plainly sets off the surface carcinoma from adjoining normal squamous epithelium and noted that the lesion advanced farther into the normal epithelium at the base than the surface thus forming an oblique junction line The emphasis on strict separation of cancerous from normal epithelium is reflected in Schiller s theory and in Meyer s theory of the development of the surface field In an excellently illustrated discussion of his observations, Schiller[110] held that the surface carcinoma expanded by the transformation of neighboring normal cells to carcinoma cells He thought it probable that the first beginning of the carcinoma was in one or more single cells and that the process occurred so rapidly that one could observe only the sharp boundary line between the already transformed surface carcinoma and the normal epithelium Meyer[80] considered the genesis of the surface field to be actual invasion of contiguous normal epithelium by the proliferating carcinomatous epithelium He construed long gently sloping oblique junction lines as evidence that the upper layers resisted invasion more than the basal layers He thought isolated dead cells at the junction line indicated that the growing cancer killed the normal epithelium as it advanced He apparently did not consider the equally acceptable thesis that they might be a part of the already established field since premature cell death is frequently seen in carcinoma in situ The observations are explained as well by the theory that a field of normal epithelium is converted to carcinoma in situ as by the theory that the field arises from progressive intraepithelial invasion by the descendants of a hypothetical single 'cancer cell

The idea that a field of normal epithelium is gradually converted to carcinoma in situ is supported by the demonstration of progressive degrees of cytologic atypism in a group of specimens and more important in even the same cervix Younge *et al*[138] TeLinde and Galvin[30, 129] and Wespi[131] have found lesser degrees of cytologic atypicality providing a gradual transition between obvious carcinoma in situ and normal epithelium These observations do not deny the occurrence of sharp junctions between patently abnormal and entirely normal epithelium described and illustrated so well by Schiller and others but do point out that a carcinoma in situ may also gradually and imperceptibly merge with the normal epithelium through a zone of less atypical epithelium Cases of the latter kind strongly suggest that carcinogenesis is a developmental progression a process of transformation of the structure and metabolism of normal cells rather than a sudden mutation in some cell whose progeny constitute the carcinoma One can

mann[34] In contrast with such well differentiated lesions, other surface carcinomas of the portio are entirely undifferentiated from basalis to surface

Other histologic features are not specially characteristic of carcinoma in situ but are generally found in endocervical ectopias An inflamed granulation tissue frequently lies beneath the atypical epithelium As illustrated in Fig 45C and D, a band of lymphocytes may quite sharply underscore the surface carcinoma (on



FIG 45 —*Carcinoma in Situ of the Portio Epithelium* A Portio epithelium normal except for slight inflammation B Basal cell hyperactivity marked by increased mitoses nuclear size and concentration in lower half upper half normal C and D Junction of carcinoma in situ on left with normal epithelium on right E and F Well differentiated carcinoma in situ prominent intracellular vacuoles and intercellular bridges in E three layered lesion in F (All photomicrographs in Figures 45 46 and 51 *are the same magnification except 46F*)

it is not obligatorily committed to such a progression Wespi's[131] systematic follow up studies clearly show that regression as well as progression of the lesion is possible To predict whether a given lesion will regress will persist or will progress to carcinoma in situ is not possible on a morphological or any other basis We must return to these practical considerations later but for the moment our interest in lesser degrees of cytologic atypism has centered on the implications for the mode of development of fields of carcinoma in situ To repeat the observations suggest a progressive neoplastic conversion of an area of normal epithelium rather than a strictly unicellular origin of the carcinoma followed by invasion of the normal epithelium

The Development of Carcinoma in Situ from Reserve Cell Proliferations and Metaplastic Epithelium —The studies of Pund and associates[99–101] Knight[66] Wespi[131] Glatthaar[41 43] and Howard *et al*[6] offer evidence that carcinoma in situ sometimes develops in epithelium derived from the endocervical reserve cells rather than from the squamous epithelium of the portio vaginalis Such an origin is seen especially in fields confined to the endocervical canal but is observed also in endocervical ectopias The carcinoma in situ may develop in an established field of metaplastic epithelium or it may arise directly from atypical reserve cell proliferation

Carcinoma in situ deriving from the reserve cells usually is histologically characterized by a crowded layer of small undifferentiated cells with fat, spindle nuclei often slanting toward the base Nuclei cut at right angles to their long axes are round Although the nuclei resemble those of reserve cells they are larger, and the chromatin is dense and granular rather than uniformly powdered Mitoses may be frequent whereas they are rarely seen in benign reserve cell hyperplasia and metaplasia The cytoplasm is scant and basophilic These cytologic differences can be seen by contrasting figure 46 E with figures 46 A and B Near the os the surface carcinoma may be better differentiated than it is higher in the canal, just as a length of metaplastic epithelium often is obviously squamous at the os but is less differentiated in the canal and in glands The junction with the portio epithelium usually is abrupt and may be vertical or sloping in either direction

The occurrence of the field in the endocervical canal is not alone sufficient evidence to establish its derivation from endocervical reserve cells because the portio epithelium can extend into that area as pointed out previously A more convincing argument is the demonstration of increasing degrees of cytologic atypicality beginning with plainly benign reserve cell hyperplasia and ending with obvious carcinoma in situ Such a series has been reported by Howard *et al*[6] and documented in their photomicrographs In a histologic study of 400 apparently normal cervices they found 14 unsuspected intraepithelial carcinomas and a group of 7 intermediary cases exhibiting metaplasia with atypicalities These cases form a chain of increasingly atypical metaplastic epithelium Although the authors segregated the cases with less severe cytologic aberrations in the group metaplasia with atypicality the dividing line between this group and intraepithelial carcinoma was admittedly subjective In that particular study the progressive development of cytologic atypicality was illustrated in a series of cases but the entire progression can be found in a single specimen For example in figure 46 C the gland on the left is filled with atypical metaplastic epithelium and the one next it on the right with in situ carcinoma the atypical metaplastic

see that Meyer's idea of carcinogenesis differs from Cohnheim's only in the hypothetical position of the aberrant mother cell—for Cohnheim a rest, for Meyer, a cell somewhere in the surface epithelium itself. This proposition once accepted, it logically follows that for Meyer the abnormal epithelium is a carcinoma whether it be confined to natural surfaces or not. The problem then becomes the proper identification of a "cancer cell" and the formulation of cytologic criteria of malignancy. A line must be drawn. On one side are all "cancer cells" and on the other side all "other cells." In this view, lesser degrees of cytologic atypism may be *mistaken* for carcinoma. As Meyer[81] said: "I showed the absurdity of the expression 'pre-cancerous' which means that the author in question cannot recognize whether a lesion is carcinoma or not." The great difficulty is that not everyone agrees *where the line is to be drawn*. Furthermore, those who would do so face a delicate problem when they are confronted with a single continuous field of progressively atypical epithelium gradually merging with the normal. Somewhere they must arbitrarily draw a line and determine that all the cells on one side are "cancer cells" and all *those on the other may be "mistaken for cancer cells."* It would seem more reasonable to think that the gradually progressive atypism mirrors a progressive derangement in the metabolism and structure of normal cells that are being converted to a carcinoma. In this view sharp distinctions between "carcinoma" and "non-carcinoma" cannot always be found during carcinogenesis. One rather seeks degrees of abnormal proliferative activity, and when an experienced observer finds sufficient abnormality he terms that stage of the process "carcinoma" according to his criteria of malignancy. We must return later to a consideration of morphological, chemical and biological criteria of malignancy.

Because imperceptibly merging degrees of cytologic atypism can be found in many cervices, almost every worker has devised his own categories or stages of atypism and labeled them in his own way. The full blown lesion that everyone would recognize is called by Younge *et al.*[138] "carcinoma in situ," by Galvin and TeLinde[3] "intraepithelial carcinoma," by Wespi[131] "increasingly atypical cervical epithelium," and by Hinselmann[5] "Rubrics III and IV" in his somewhat cryptic system. Lesser degrees of atypicality have been termed by Younge *et al.*[138] "anaplasia ranging to equivocal carcinoma in situ," by TeLinde and Galvin[1 9] "basal cell hyperactivity," and by Wespi and Mueller[83] "irregular epithelium." The gap between Hinselmann's Rubrics II and III is so great that one cannot find a comparable state of activity in his system. All these authors seem to agree that only these lesser degrees of atypism are encountered in some cervices *but all find instances in which a field of full blown carcinoma in situ merges with such areas.* It is not possible to precisely match the several nomenclatural systems. In a general way, however, all call attention to epithelium which departs from normal but exhibits only some of the atypicalities which have been detailed for carcinoma in situ. The nomenclature of Galvin and TeLinde[35] especially points to the early changes occurring in the basal layer of the epithelium while the upper layers are yet unaltered (Fig. 45B). "Anaplasia" presumably refers to maturation defect, sometimes called dedifferentiation or cataplasia. When Younge *et al.*[138] further speak of "three grades of anaplasia," they *undoubtedly are trying to catalog the varying degrees of atypism* found in this epithelium. Although the association of this kind of epithelium with carcinoma in situ in the same cervix inculpates it as a stage in carcinogenesis

epithelium was found to merge nearby with benign metaplasia like that shown in figure 46 B A case I have recently studied is of special significance because two morphologically distinct types of carcinoma in situ were present in the same cervix and each type could be traced to a distinct type of increasingly atypical metaplastic epithelium

Whether such a carcinoma in situ appears in the canal or in an ectopia, the field almost invariably covers the surfaces of endocervical glands as well as the topographical surface This is expected because the reserve cells are found beneath the glandular and the surface columnar epithelium The columnar cells may no longer be present in some places but in the glands they may cover the carcinoma in situ Since the glandular lining is a natural surface, the carcinomatous layer in this position is no less in situ than it is on the topographical surface It will be recalled that the endocervical glands often extend deeply into the wall of the cervix Instances like that shown in figure 46 F, in which a carcinoma in situ involves glandular surfaces seem to be often mistaken for genuinely invasive neoplasms, if one can fairly judge from photomicrographs

The area just inside the external os is most often involved by surface carcinoma of this type but the field may extend onto an ectopia or in the other direction to the surface of the uterine corpus The field may transform the entire endometrial surface into a granular sugar icing coat but cases of this kind are not common Extension of the field into the Fallopian tubes is rare

A mixed mononuclear inflammatory infiltrate quite like that seen with carcinoma in situ of the portio vaginalis frequently develops It is hardly possible to know whether the inflammatory reaction appears before or after the epithelial lesion is established but it occurs without invasion of the stroma or significant necrosis of the epithelium The inflammation therefore cannot be construed as a protective device It seems if anything to be harmful as we shall note later in discussing the development of stromal invasion within the field

It is appropriate to record here the vigorous denials that cervical carcinoma ever arises from the subcylindrical basal cells The most categorical criticisms of the thesis are found in Meyer s writings from which the following quotations can be taken as illustrative

A study of very early squamous cell carcinomas leads to the conclusion that they arise always from squamous epithelium and always from the basal cells In most cases at the margin of an old erosion [80]

carcinoma does not arise from active epidermization undergoing proliferation as in erosion healing [80]

One might conclude then that carcinoma and epidermization of erosion are two distinct and unrelated conditions They both arise from the basal cells of the squamous epithelium at the margins of erosions But epidermization is a benign restoration and when completed approximates the normal Carcinoma does not arise directly from it [80]

Novak[91] would seem to agree with Meyer although he cautions against mistaking atypical epidermization for carcinoma

Schiller[110] also denied that squamous carcinoma often arises from the islands of epithelium trapped in ' erosion healing He stated that the early carcinomas he studied sprang entirely from the superficial squamous epithelium bordering the cylindrical epithelium

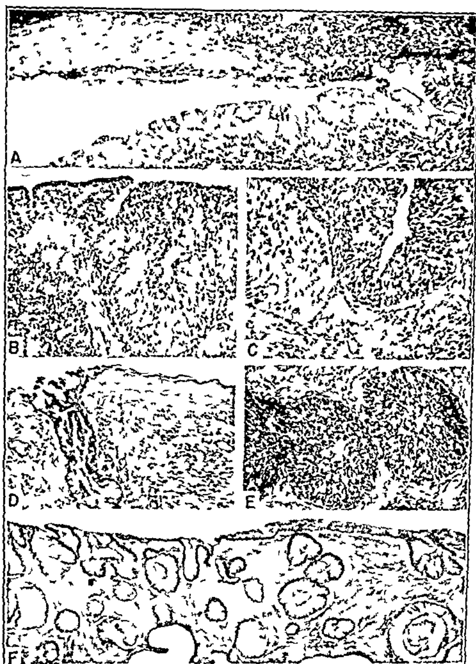


Fig. 46 – *Carcinoma in Situ in Metaplastic Epithelium* A Beneath the columnar epithelium benign reserve cell hyperplasia below and squamous metaplasia above B Irregular festoons of benign proliferating reserve cells contrasted with C D and F C Squamous metaplasia with cytologic atypicalities in gland on left carcinoma in situ in gland on right D Benign regenerating portio epithelium in the transformation zone of an endocervical ectopia E Typical undifferentiated carcinoma in situ almost filling endocervical gland *F Carcinoma in situ on surface* and lining endocervical glands deep in the cervix a pattern that may be mistaken for invasive carcinoma

what less than the incidence in cervices in general, but the occurrence is not unknown

The conclusions of Carmichael and Jeaffreson regarding the relationship between metaplasia and carcinoma have not always been accurately reiterated After commenting that a lesion so common as metaplasia obviously could not be malignant they agreed that it might be precancerous but went on to say

'In most cervical cancers it is impossible to determine with certainty whether the growth has arisen in the squamous epithelium or in the endocervical mucosa much less to trace its origin to a small patch of previously altered epithelium Probably the metaplastic epithelium does occasionally give rise to malignant growths but it is clearly impossible to estimate the frequency of this occurrence or to ascertain whether the atypical [i e metaplastic] epithelium is more prone to undergo malignant degeneration than the normal epithelium [9] Presumably, their studies were not augmented by comparative histologic material from cases of carcinoma in situ Anderson s comments indicate that cervical carcinoma in situ was not under intensive study in England at that time If the entire cervix is available for study one is usually able to trace the origin of the lesion to transformation of a field of squamous portio epithelium or metaplastic epithelium There are nevertheless instances in which one cannot conclusively demonstrate the histogenesis from either portio epithelium or reserve cells In such cases the field of the surface carcinoma usually occupies both canal and face appearing to derive from reserve cells in the canal and from portio epithelium on the face A parallel can of course be drawn with cases of epidermization and metaplasia in which the squamous epithelium about the external os is so well differentiated that one cannot decide whether it represents in growth of portio epithelium or well differentiated metaplastic epithelium

Epithelial Hyperplasias to be Distinguished From Carcinoma in Situ — Several varieties of epithelial hyperplasia should be segregated because they can be confused with carcinoma in situ Even though these lesions are sometimes associated with carcinoma in situ they are so frequently encountered apart from it that one can conclude they usually are benign From the viewpoint of carcinogenesis they usually are reversible or non progressive reactions and so at most are only facultatively precancerous

First should be mentioned benign reserve cell hyperplasia and squamous metaplasia of the endocervix quite common lesions that have already been discussed As illustrated by Carmichael and Jeaffreson [9] peculiar festoons of metaplastic epithelium roll in and out of the stroma and may be mistaken for in situ or invasive carcinoma The resemblance to invasive carcinoma is illustrated in figure 46B where glands lined with metaplastic epithelium extend irregularly into the underlying stroma Although the cells in the metaplastic epithelium are irregularly disposed as in carcinoma in situ the cell population is not so dense More important metaplastic epithelium (Figs 46A and B) is distinguished from carcinoma in situ (Fig 46 E) by absence of significant cytologic atypicalities The nuclei are small regular and homogeneously filled with finely granular or lightly stippled chromatin Mitoses are seldom seen This superficial resemblance to carcinoma and the differential diagnosis have been carefully discussed in several papers [73 89 90 1 8]

The earliest shades of atypical metaplasia usually are not striking [6] They are marked by cells with somewhat larger vesicular round nuclei only lightly

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In discussing their histogenetic theory, Galvin and TeLinde[35] say, 'Our *observations lead us to believe that cervical* carcinoma begins in the basal layer of the portio vaginalis and that slight changes in this layer may be the precursor of intraepithelial carcinoma "

Younge *et al*,[128] apparently agree

'Carcinoma in situ replaces the columnar cells of the endocervix or the endo cervical glands as it advances *This is in contrast to the undergrowth which takes* place in squamous metaplasia or epidermatization The benign undergrowth of squamous metaplasia as distinguished from the total replacement of the endo cervical epithelium by carcinoma in situ was first noted over twelve years ago, and no exception to the rule has been found to date '

It is universally recognized that squamous metaplasia is common in endo cervical polyps but carcinoma in situ and invasive carcinoma seldom arise in polyps

The available evidence does not however, warrant the conclusion that a field of metaplastic epithelium or reserve cell hyperplasia never gives rise to a car cinoma in situ More confusion than is necessary has arisen from substituting ' never gives rise for ' seldom gives rise When one considers that reserve cell hyperplasia or metaplasia is present in about 80 per cent of all cervices, it becomes at once apparent that this process does not inevitably lead to carcinoma in situ Metaplastic epithelium does not always become cancerous neither does portio epithelium which constantly replaces itself from the basal layer as any other epithelial surface does In commenting that transitions from benign epidermiza tion to carcinoma are unknown Meyer[80] said that carcinoma develops at the margin of not within, the erosion area yet he also said that he had studied a case arising inside an erosion Furthermore Rubin[10] had made it clear that serial sections* of his case II revealed no connection between the squamous epithelium *and a focus of surface carcinoma in a gland Meyer,*[80] *in fact gave an excellent* morphological description of atypical reserve cell hyperplasia in his account of studies with Garlach in which they discovered carcinomas in situ in endocervical biopsies when the portio was usually normal These studies seem not to have altered his previous conclusions that cervical carcinoma develops only in the squamous epithelium[81]

Although the carcinoma in situ seems usually to displace the columnar epithel ium there are cases in which part of the field is found beneath intact columnar cells Rubin[10] illustrated such lesions in his article of 1910 In Figure 51A one can see a cross section of a gland almost filled by surface carcinoma, yet the over lying columnar epithelium remains intact (An invasive bud has broken out of the field in the upper right corner as we shall note later)

Statements that polyps do not become carcinomatous would be more accurate if modified to say that they seldom become carcinomatous We have recently studied a carcinoma in situ arising in the metaplastic epithelium of an endocervical polyp the remainder of the canal and the portio vaginalis were uninvolved by the lesion *Reporting 459 polyps* Knight[66] found metaplasia in 53 of which 2 cases were malignant Fluhmann[9] reported only 2 carcinomas arising in polyps It would seem that the incidence of in situ and invasive carcinoma in polyps is some

* In a personal communication to the writer Dr Rubin[9] stated the detail of his technique Sagittal blocks of the entire cervix were serially sectioned in paraffin at 5 to 7 micra thickness every fifth section being retained for study

on occasion extends well beyond the transformation zone to the periphery of the portio vaginalis and uncommonly even to the vagina

There may, however be no signs or symptoms of the lesion This is especially likely if the field is confined to the canal Lesions of this type usually derive in my opinion, from reserve cell hyperplasia and metaplasia These lesions frequently occupy the linings of endocervical glands and may, therefore extend deeply into the cervix without however genuinely invading the stroma Rarely, the field may cover the entire endometrial surface

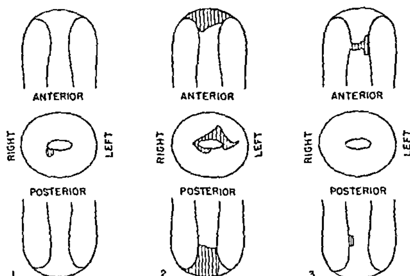


FIG 47 —Diagram illustrating the variation in size and anatomical location in cases of cervical carcinoma in situ Area of surface field indicated by vertical lines (Adapted from Foote and Stewart[31])

Obvious cytologic atypicality like that of carcinoma	PORTIO VAGINALIS AND ENDOCERVIX Carcinoma in situ (and synonyms) and surface carcinoma at margin of obvious invasive carcinoma	
Evident but less severe cytologic atypicality sometimes progressive to carcinoma in situ	PORTIO VAGINALIS Irregular epithelium[82] Anaplasia[88] Basal cell hyperactivity[125] Hyperactive epithelium[1] Precancer cell complex[5]	ENDOCERVIX Squamous metaplasia with atypicality[62]
Cytologic atypicality not like that of carcinoma usually benign	PORTIO VAGINALIS Regenerating portio epithelium in a transformation zone Simple atypical epithelium[88] Leukoplakia Condyloma acuminatum	ENDOCERVIX Reserve cell hyperplasia and squamous metaplasia[8,9,62] Simple atypical epithelium[131]

FIG 48 —Simplified chart of varieties of abnormal surface epithelium found in the cervix uteri

stippled with chromatin small nucleoli and somewhat more abundant cytoplasm which may be acidophilic, basophilic or vacuolated Mitoses are seen but are scarce This type of epithelium has been called by Wespi[131] "simple atypical epithelium" and he considers it usually harmless It is often seen in transformation zones near the external os

Deviations from normal of regenerating and hyperplastic portio epithelium in transformation zones are also frequently encountered The squamous epithelium at the margin of a pseudoerosion often contains little glycogen, and its lower third or half is made up of cells with distinctly basophilic cytoplasm Occasional mitoses are found in the basal row Although these changes merge with the "basal cell hyperactivity" of TeLinde and Galvin illustrated in figure 45B, they alone only characterize regenerating epithelium in which there is active synthesis of nucleic acid When irregular pegs of such epitheluim dip into the granulation tissue of an ectopia, the picture may at first glance, however, resemble carcinoma in situ

The cervical leukoplakias or white spots, usually are benign Unfortunately diverse usage of the term "leukoplakia" has caused almost unending confusion A number of histologic alterations of the squamous epithelium may appear as white areas These include benign proliferating portio epithelium lacking glycogen and thick, parakeratotic or keratotic epithelium resembling exposed epithelia as well as obvious in situ carcinoma In addition Hinselmann has used the term to cover a wide range of colposcopic abnormalities not readily appreciated by simple inspection It is not easy to translate his Rubrics I II III and IV into conventional histologic description, but lesions with cytologic atypism in III and IV he considers the same as the characteristic surface carcinomas of Meyer Schiller and von Franque[55] Martzloff[70] and Schiller[113] have pointedly discussed the variation in histologic structure of leukoplakia As Schiller proposed it would seem best that the term "leukoplakia be discarded except as a description of any white gross lesion the exact nature of which can be determined only by histologic study *Although a few heaped up and keratotic in situ carcinomas have* a dull white appearance, the usual leukoplakia is quite without cytologic atypism

Acuminate condylomas like those frequently found on the external genitalia occasionally arise on the cervix There are also squamous papillomas and glandular polyps that develop during pregnancy These benign lesions can be identified histologically

Recapitulation The Signs, Symptoms and Anatomic Location of Carcinoma in Situ of the Cervix Uteri —It is now possible to summarize the previous discussion and to consider the signs symptoms and anatomic location of carcinoma in situ in terms of its pathogenesis

The lesion frequently develops in the transformation zone of an endocervical ectopia and therefore is often found in an inflamed hyperemic cervix Only slight inflammatory changes may be found in a circumoral vermilion zone but the development of ulceration causes a striking inflammatory reaction Beneath a more or less well epithelialized surface lie gray or tan crypts of endocervical glands Because of inflammation and edema the surface epithelium is fragile and if it is even slightly disturbed bleeding may result Some form of abnormal bleeding including post coital spotting is reported in one fourth to one half of all cases of carcinoma in situ The field usually is adjacent to the external os but

on occasion extends well beyond the transformation zone to the periphery of the portio vaginalis and uncommonly even to the vagina

There may, however be no signs or symptoms of the lesion This is especially likely if the field is confined to the canal Lesions of this type usually derive in my opinion from reserve cell hyperplasia and metaplasia These lesions frequently occupy the linings of endocervical glands and may, therefore extend deeply into the cervix without however genuinely invading the stroma Rarely the field may cover the entire endometrial surface

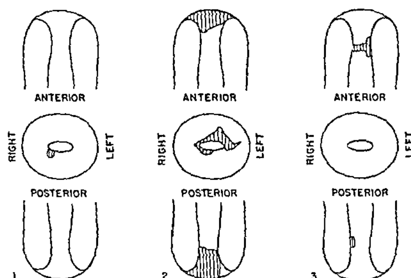


Fig 47 —Diagram illustrating the variation in size and anatomical location in cases of cervical carcinoma in situ Area of surface field indicated by vertical lines (Adapted from Foote and Stewart [31])

Obvious cytologic atypicality like that of carcinoma	PORTIO VAGINALIS AND ENDOCERVIX Carcinoma in situ (and synonyms) and surface carcinoma at margin of obviou invasive carcinoma	
Evident but less severe cytologic atypicality some time progressive to carcinoma in situ	PORTIO VAGINALIS Irregular epithelium [3] Anaplasia [1, 8] Basal cell hyperactivity [1, 2] Hyperactive epithelium [117] Precancer cell complex	ENDOCERVIX Squamous metaplasia with atypicality [62]
Cytologic atypicality not like that of carcinoma usually benign	PORTIO VAGINALIS Regenerating portio epithelium in a transformation zone Simple atypical epithelium [1] Leukoplakia Condyloma acuminatum	ENDOCERVIX Reserve cell hyperplasia and squamous metaplasia [3, 61] Simple atypical epithelium [3]

Fig 48 —Simplified chart of varieties of abnormal surface epithelium found in the cervix uteri

Some of the variants in anatomical distribution are illustrated in figure 48, which has been adapted from Foote and Stewart.[31] The few examples selected suggest the great range in extent and position of fields of carcinoma in situ that are encountered. Wespi,[131] as well as Foote and Stewart,[31] has reported the occurrence of multiple, discontinuous fields in a given cervix.

Finally, some investigators have found gradually progressive atypicalities developing in the portio epithelium and also in metaplastic epithelium. This would seem to indicate that a field of epithelium is gradually transformed into a field of carcinoma in situ. At exactly what point these atypical hyperplasias become irreversibly neoplastic cannot be determined or predicted in a given case but certain hyperplastic changes in the cervix are seen so frequently that one can be certain they usually are self limited and not precancerous in an obligatory sense. The gamut of epithelial changes found in the cervix are shown in the following diagram modeled after Wespi[131] and Glatthaar.[43]

THE RELATION OF CARCINOMA IN SITU TO INVASIVE CARCINOMA OF THE CERVIX UTERI

The Evidence That Invasive Carcinoma May Develop in Fields of Carcinoma in Situ.—*Cytologic Evidence.*—It has already been pointed out that the cytologic atypicalities found in cases of carcinoma in situ much resemble those found in obviously invasive carcinoma. These atypicalities have been offered as prima facie evidence that carcinoma in situ is already a malignant neoplasm. Most of these abnormalities are, however, duplicated by rapidly growing and dividing cells in other situations. The proliferating epithelial cells at the margin of a chronic dermal ulcer may exhibit increased mitotic activity, prominence of nucleoli and basophilia of cytoplasm, yet the hyperplasia usually does not progress to neoplasia. When it does, moreover, it seems doubtful that one can define precisely the changes that identify "cancer cells" as opposed to "hyperplasia cells." Rapidly proliferating fibroblasts in the base of an inflammatory ulcer regularly exhibit similar atypia, yet they do not become sarcomas. This old argument need not be pursued in detail. Conservative opinion holds that rigorous criticism will not admit the identification of isolated "cancer cells" with the microscope. Neoplasia is considered a process that is identified not by cytologic abnormalities but by abnormal relationships between cells and tissues. Even then the designation "carcinoma" is in some sense a prediction based on experience that this abnormality will progress unabated. Study of endometriosis of the colon or of normal placentation, for example, provides sobering reflection about the indisputable histologic criteria of malignancy.

Surface Carcinoma at the Margin of Invasive Carcinoma.—Although the cytologic abnormalities alone do not define carcinoma in situ as a neoplasm, this surface lesion seems ominous because it is sometimes found at the margins of an already invasive squamous carcinoma. Schiller[111] cited Schottlaender and Kermauner's[116] description of such a peripheral surface coat made up of cells which were, according to Schiller, morphologically indistinguishable from the cells of the invasive neoplasm. A reasonable conclusion was that merging surface component and invasive component were all one carcinoma. That same picture had also been seen previously by Cullen,[17] and in his monograph it is beautifully illustrated by

Max Brodel and Hermann Becker Cullen spoke both of ' transformation ' of adjoining surface epithelium and of extension of the carcinoma to adjoining surfaces In his studies of the development of cervical carcinoma Schiller[11] found however, that the size of the invasive component of the field was inversely related to the size of the surface field He found a few genuinely invasive tumors only a few millimeters in dimension planted in extensive contiguous fields of surface carcinoma These observations can be confirmed if one has opportunity to study small Stage I unirradiated carcinomas removed by radical hysterectomy A suggested interpretation is that the invasive neoplasm has arisen in a previously established field of carcinoma in situ rather than that the superficial carcinomatous coat has spread outward from the invasive tumor Before completing this argument let us turn for historical reasons to certain clinico pathologic case studies supporting the theory that infiltrating squamous carcinoma may develop from in situ carcinoma

Case Studies —In the past twenty five years there has been recorded a limited number of cases in which an invasive squamous carcinoma arose in a cervix known by previous biopsy to harbor carcinoma in situ Cases of this kind materialize in several ways In earlier days when carcinoma in situ was neither widely recog nized nor accepted it sometimes passed undetected in biopsies of ' chronic cer vicitis or ' pseudoerosion Hence upon review of former cervical biopsies it was possible to find in retrospect previously unrecognized cases Some observers systematically reviewed a series of former biopsies others only reviewed earlier biopsies of a patient or patients presenting an invasive carcinoma Additional case material of this nature has come from patients who refused treatment even though apprised of the in situ carcinoma Only a rare case has been deliberately observed without treatment Table VII catalogs reported cases of these kinds

These studies imply that carcinoma in situ is a biological predecessor of inva sive carcinoma and that the in situ stage may be present in some cases for years before invasive carcinoma ensues This is consistent with the fact that the average age of patients with demonstrated in situ carcinoma is about ten to twelve years less than the average age of patients with demonstrated invasive carcinoma Figure 50 graphically shows the age distribution by five year periods of 3 series of cases of in situ carcinoma compared with a similar composite curve derived from data reported in 6 series of invasive carcinoma totaling more than 7000 cases This particular age relationship offers no information concerning the percentage of cases that become invasive nor does it alone guarantee a necessary relationship between in situ and invasive cervical carcinoma

The conclusion that invasive followed in situ carcinoma in these cases can be questioned One can object that the biopsy specimen may have sampled only the marginal carcinomatous surface about an invasive carcinoma present all the time By these studies alone the objection cannot be countered it may be true in some cases and one might choose to think that if every cervix purported to hold a carcinoma in situ were sufficiently sectioned an invasive lesion would invariably be discovered In short one could adopt the thesis that marginal extension from an invasive carcinoma is the proper explanation of every field of carcinoma in situ However this thesis is not substantiated by my experience for even serial sections fail to reveal any invasive focus in some cases of carcinoma in situ In other words genuinely in situ carcinoma of the cervix does occur

TABLE VII.—CASES OF CARCINOMA IN SITU PROGRESSING CLINICALLY TO INVASIVE CARCINOMA

Author	*Case No*	*Carcinoma in situ present at age*	*Carcinoma in situ demonstrated by*	*Invasive Carcinoma demonstrated by*	*Interval before invasion demonstrated*	*Manipulation etc in interim*
Anderson, A. F.[2]	1	40	Biopsy	Gross examination	7 10/12 yrs	Cauterization
	2	58	Biopsy	Gross examination	3 11/12 yrs	One biopsy
	3	43	Curettings	Biopsy	1 3/12 yrs	Nothing
Galvin[36,37]	1	47	Biopsy	Biopsy	7 9/12 yrs	Nothing
	2	39	Curetting	Biopsy	3 6/12 yrs	Nothing
	3	32	Biopsy	Biopsy	4 7/12 yrs	Nothing
	4		Biopsy *	Biopsy	4 10/12 yrs	
	5	40	Biopsy	Biopsy	6 7/12 yrs	Subtotal hysterectomy
	6	57	Biopsy	Biopsy	14 10/12 yrs	Nothing
	7	32	Curetting	Biopsy	9 11/12 yrs	Nothing
	11	44	Biopsy	Biopsy	5 8/12 yrs	Nothing
	15	44	Biopsy	Biopsy	1 yr	Nothing
	18	50	Biopsy	Biopsy	7 yrs	Nothing
	21	37	Biopsy	Biopsy	16 8/12 yrs	Cauterization
Galvin and TeLinde[38]				Autopsy	0 6/12 yrs	Irradiation
Goldberger and Mintz[45]	2	49	Biopsy	Biopsy	5 10/12 yrs	Nothing
Hinselhorst[44] and Limmrich[45]	Frau Ko	31	Serial blocks of amputated portio	Biopsy histologic examination of hysterectomy specimen	About 5 yrs	Portioamputation
Hinselmann	Alpers	24	Amputation	Operative specimen	About 4 yrs	Nothing
Jones[4]		46	Biops	Cone Biopsy	12 7/12 yrs	Repeated cauterization electroconization and biopsies

Knight[46]	1	55	Polypectomy	Biopsy	5 yrs	Inadequate doses of radium
	8	37	Curettage	Clinical	Died after 4 yrs	5000 mg hrs radium x ray
Payne			Curettage Amputation	Cervical epithelioma (Stage II)	37 yrs	
Pund et al		33	Biopsy		4 7/12 yrs	Nothing
Rubin[98]	3	55	Amputation	Biopsy	5 4/12 yrs	x ray
Scheffey[19]			Cervical Tissue	Stage III carcinomatous lesion	9 yrs	Nothing
Schiller[41]	Anna J	4	[illegible]	Hysterectomy *	1 4/12 yrs	Irradiation of pituitary Curettage
	Elizabeth S	38	Biopsy	Hysterectomy	9 yrs	Nothing
	Antonie N	59	Biopsy and Hysterectomy	Biopsy	Not given	x ray radium
	Marie J [3, 14]	45	Biopsy and Hysterectomy	Presumably autopsy[14]	Not given	Nothing
Schmitz and Benjamin[25]	Mrs S		Amputation	Biopsy	0 10/12 yrs	Nothing
Smith and Pemberton[2] †	1†† Path No 6123	25	Right sided Trachelorrhaphy	Biopsy	4 1/12 yrs	Nothing

Only basal cell hyperactivity demonstrated

Although the case was considered carcinoma by experienced observers the illustration (Emmrich[25] Figure 3 p 4) shows only glandular involvement This case should probably be placed in Table VIII p 242

* In my opinion there is no convincing illustration of genuine invasion Both cases should probably be placed in Table VIII p 242

† Dr Paul A Younge has reviewed these data and supplied additional information in a personal communication of November 1951 Certain published data including pathology numbers of cases have been corrected by Dr Younge Case 15 in Smith and Pemberton's[12] series is no longer accepted by Dr Younge

†† Previously reported by Pemberton and Smith[91]

TABLE VII.—CASES OF CARCINOMA IN SITU PROGRESSING CLINICALLY TO INVASIVE CARCINOMA.—(*Continued*)

Author	*Case No*	*Carcinoma in situ present at age*	*Carcinoma in situ demonstrated by*	*Invasive Carcinoma demonstrated by*	*Interval before invasion demonstrated*	*Manipulation etc in interim*
	2 Path No 5690	38	Trachelorrhaphy		4 9/12 yrs	Nothing
	15†	34	Trachelorrhaphy	Biopsy	12 6/12 yrs	Nothing
	16 Path No 14565	28	Trachelorrhaphy cauterization	Pathological examination	6 1/12 yrs	Presumably nothing
	Path No 16494		Biopsy	Biopsy	3 1/12 yrs	Radium
Stevenson and Scipiades[18]	2	78	Trachelorrhaphy	Biopsy	8 8/12 yrs	Nothing
	13	34	Biopsy	Autopsy	3 yrs	Biopsy Curettage of canal
Wespi[1]	47	45	Biopsy	Histologic examination	3 2/12 yrs ‡	Nothing
	45	59	Biopsy	Biopsy	10 3/12 yrs	Cauterized repeatedly
	27	23	Biopsy	Biopsy and Hysterectomy‡‡	8 11/12 yrs	Nothing
Younge[38]†	Path No 18737		Biopsy		3 4/12 yrs	Cauterization twice
Younge, Hertig and Armstrong[1]*†	Path No 27434		Biopsy	Biopsy	0 11/12 yrs	One biopsy

‡ See German text for this datum. The English translation is incomplete.
‡‡ No genuine invasion found by the author. The case should probably be placed in Table VIII, p. 242.

To grant the authenticity of genuinely in situ carcinoma and of genuinely invasive carcinoma but to propose that there is no necessary relationship between the two lesions in the same cervix is a somewhat different critical twist. Indeed serial clinico pathologic observations do not exclude the possibility that an independent invasive tumor unrelated to the previous carcinoma in situ may have developed in the course of several years. But the demonstration of genuinely invasive microscopic buds within fields of carcinoma in situ is the most conclusive evidence of an undeniable relationship between the two lesions in some cases even though no obligatory relationship can be guaranteed in cases such as those listed in Table VII.

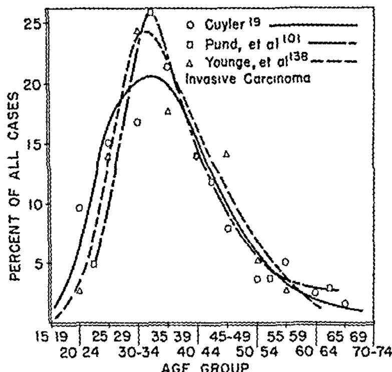


FIG 49 —Age distribution of three series[19, 101, 138] of cases of cervical carcinoma in situ contrasted with that of invasive cervical carcinoma. The per cent (or calculated per cent) of cases falling in each five year age group is plotted as a point. For invasive carcinoma a composite curve has been derived from data in six series.[19–48]

Microscopic Invasive Foci. A satisfactory histological demonstration of genuinely invasive microscopic buds within the field of a carcinoma in situ most nearly convinces even the quite critical observer of the pathogenicity of the surface field. It will be recalled that Schiller found ever smaller gross tumors in extensive surface fields but it has already been suggested that one might interpret the invasive lesions as primary though they are only a few millimeters in dimension. This interpretation is less reasonable however when no gross tumor is present anywhere in the field but only invasive buds of a few cells such as those demonstrated in Figure 51. Furthermore by means of serial sections of entire cervices multiple invasive buds have been demonstrated in a single field of carcinoma in

TABLE VII —CASES OF CARCINOMA IN SITU PROGRESSING CLINICALLY TO INVASIVE CARCINOMA —(*Continued*)

Author	*Case No*	*Carcinoma in situ present at age*	*Carcinoma in situ demonstrated by*	*Invasive Carcinoma demonstrated by*	*Interval before invasion demonstrated*	*Manipulation etc in interim*
	2 Path No 5690	38	Trachelorrhaphy		4 9/12 yrs	Nothing
	15†	34	Trachelorrhaphy	Biopsy	12 6/12 yrs	Nothing
	16 Path No 14565	28	Trachelorrhaphy cauterization	Pathological examination	6 1/12 yrs	Presumably nothing
	Path No 16494		Biopsy	Biopsy	3 1/12 yrs	Radium
Stevenson and Scipiades[30]	? 13	28 34	Trachelorrhaphy Biopsy	Biopsy Autopsy	8 8/12 yrs 3 yrs	Nothing Biopsy Curettage of canal
Wespi[33]	47	45	Biopsy	Histologic examination	3 2/12 yrs ‡	Nothing
	45 27	59 23	Biopsy Biopsy	Biopsy Biopsy and Hysterectomy ‡‡	10 3/12 yrs 8 11/12 yrs	Cauterized repeatedly Nothing
Younge[184]†	Path No 18737		Biopsy		3 4/12 yrs	Cauterization twice
Younge, Hertig and Armstrong[1,84]†	Path No 21434		Biopsy	Biopsy	0 11/12 yrs	One biopsy

‡ See German text for this datum The English translation is incomplete
‡‡ No genuine invasion found by the author The case should probably be placed in Table VIII p 242

larger nucleus. Often such an accumulation of differentiating cells arises in the midst of the carcinomatous layer as seen toward the left of figure 51 B, and apparently will remain there without further development but where it originates nearer the basal layer the pseudopearl may become an invasive bud. Then one can see the better differentiated, squamous cells lying in juxtaposition to stroma without an intervening layer of basal cells. Parenthetically, the advancing margin



Fig. 51. *Microscopic Invasive Buds.* A. Carcinoma in situ arising from reserve cell proliferation beneath the columnar epithelium of a gland (above right); a better differentiated invasive bud irregularly invading loose stroma (above left); possible invasion. B. Carcinoma in situ of the portio (lower right); a better differentiated invasive bud (left); a non-invasive intraepithelial pseudopearl. C. Right, carcinoma in situ filling a gland; an undifferentiated forked invasive bud projecting upward and cluster of invasive carcinoma (left).

situ[127] Some illustrative patterns of invasion I have found in serial section studies are shown in Figure 52 It is hardly reasonable to suppose for example, in Case No 1 Figure 50 that 51 minute, invasive buds resembling the one shown in Figure 51 A arose independently in the same cervix and then by extensive mar*ginal proliferation joined to establish the surface carcinoma* The point need not be labored Within the field of the carcinoma in situ multiple invasive foci surely developed

It can be seen that there is great variation in the pattern and distribution of invasive foci within different fields The surface field in Case 2 was as extensive as in Case 1 yet only two microscopic invasive foci were found In Case 3, on the contrary the surface field was an area of only a few square millimeters but when invasion occurred tongues of carcinoma quickly penetrated lymphatic channels and extended to all four quadrants of the cervix

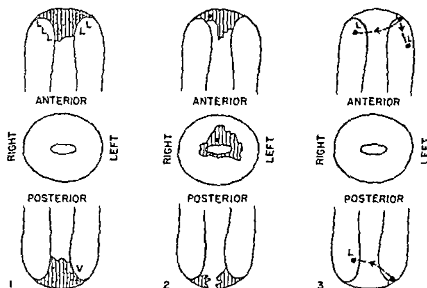


Fig 50 — Diagram illustrating the variation in patterns of microscopic invasion found by serial sections in fields of cervical carcinoma in situ Area of surface field indicated by vertical lines invasive foci by dots lymphatic penetration by L venous invasion by V In Case No 3 arrows indicate paths of lymphatic invasion

The criteria by which genuine stromal invasion is recognized have not been critically set forth My colleagues and I have been specifically interested in identifying true invasion within fields of cervical carcinoma in situ that have been serially sectioned There are several certain and a few doubtful modes of invasion In the upper right corner of figure 51 A a bud of a few cells better differentiated than those *in the surrounding field has pushed irregularly outward from the basal* layer into an area of loosened stroma The invading cells have larger and more variable nuclei and have acquired more cytoplasm than the cells occupying the surface field along the lower rim of the gland Similar differentiated invasive buds also develop in fields of carcinoma in situ arising in portio epithelium Such an invasive focus is *illustrated in the lower right corner of figure 51 B* Elsewhere in this field were found similar intraepithelial pseudopearls clusters of a few polyhedral cells that acquire a more abundant cytoplasm and perhaps a little

carcinoma is indistinguishable from the non invasive portion The invasive part usually is somewhat better differentiated the surface part more anaplastic In the invasive part the cells have more cytoplasm and tend to be somewhat larger and more variable the nuclei are somewhat more vesicular and the nucleoli are larger We have just noted that this is often the case with the earliest invasive buds as well as with the more advanced invasive growths Perhaps this is the reason that Nieburgs and Pund[97] have observed more variability among cells exfoliated from invasive carcinoma than among those from pre invasive carcinoma

In contrast with the usual patterns of buds tongues clusters or masses of invading cells one may rarely find invasion by separate cells that seem to flake off into the stoma An illustration of this pattern of invasion can be found in one of Novak s papers * The same pattern found at the margin of an obvious, deeply invasive carcinoma is readily understood but the interpretation is most difficult if only a few cells seem to be separated from the stratum basalis of an in situ carcinoma The difficulty is made greater if the underlying connective tissue has been severely disturbed by inflammation This particular mode of early invasion is interesting because certain experimental studies [6] have suggested that invasiveness of cancer may be caused in part by the lack of adhesiveness and by the amoeboid activity of cells from carcinomas In my experience however early invasion by disjoined cells is much less common than by cohesive clusters like those illustrated in figure 51

In addition to the various patterns that mark genuine stromal invasion, there are some configurations that may be misinterpreted as invasion Carcinomas in situ arising in the portio epithelium of a transformation zone present confusing pictures especially if there is much underlying inflammatory reaction In many benign transformation zones irregular tongues of regenerating portio epithelium dip into the stroma for reasons already mentioned When epithelium of this kind is converted to in situ carcinoma these irregular tongues may be mistaken for early invasion Usually no problem arises if inflammation has not disrupted the limiting stromal membrane but even then a decision is occasionally difficult However irregular contour of the rete pegs is characteristic of epithelium regenerating over granulation tissue in any site and although the relationships may be confusing is hardly acceptable evidence of stromal invasion If it is accepted then invasion will erroneously be described in many cases of carcinoma in situ of the portio vaginalis

Sections passing other than perpendicular to the surface likewise present problems in proper interpretation At angles of about 45 degrees the pegs are lengthened and pointed and fade into a streaming stroma Similarly the interdigitating stromal cones are lengthened or they are often disguised as apparently circumscribed islands in the middle and upper layers of the epithelium (Fig 45 D) Cuts parallel with the surface produce almost perfectly round islands of epithelium which are composed of only small basal cells at the lower tips of pegs All these configurations recall the conic sections of solid geometry so often demonstrated in schoolrooms with wooden models

It has already been mentioned that endocervical glands filled with surface carcinoma must be recognized and distinguished from genuinely invasive carcinoma No matter how cut they are usually round regular masses sharply

* See Novak Figs 4 and 5

of an obviously invasive carcinoma usually shows similar squamous cells pushing into surrounding stroma. One might, however, doubt that these configurations are truly invasive, but from tiny buds of this kind long thin tongues may extend many micra through the stroma and may occasionally invade lymphatic channels and, rarely, small blood vessels.[1, 7]

The stromal changes at these sites of invasion are as remarkable as the epithelial changes. In the normal cervix there usually is no hyaline basement membrane, but only a layer or two of flattened, delicate strands of collagen separate epithelium from underlying stroma. Just at the points of invasion described in figure 51 A and B, this layer and the underlying collagenous fibers are loose and widely separated, the epithelial buds pushing into almost empty appearing spaces. A moderate lymphocytic infiltrate can be seen, sometimes many lymphocytes concentrate at the invasive focus, the epithelial cells extending into a pool of inflammatory cells. What part, if any, the gathering lymphocytes have in altering the stroma is not known, but they certainly do not cause necrosis of the invading cells. There is no detailed information available about these stromal changes in cervical carcinoma. Since one comes upon such invasive foci only by chance, detailed histochemical investigation of the alterations would be difficult. Perhaps some enzyme, such as hyaluronidase, is concentrated at the site and alters the ground substance. Fisher and Hollomon[2] have suggested that a critical number of cancer cells might be necessary to initiate a malignant growth because several cells could more likely alter the chemical environment than could a single cell. One might speculate that this "critical size concept" of the origin of cancer foci better fits the origin of invasive buds and accompanying stromal changes in a field of altered epithelium than it does their idea that cancers arise de novo from the chance bunching together of several mutant cells in normal epithelium.

Another type of genuinely invasive bud, illustrated in figure 51 C, differs from that just described. It is not a differentiated pseudopearl but is a bud composed of entirely undifferentiated basal cells. It could not be recognized in a field of portio in situ carcinoma with irregular pegs extending into an inflamed stroma but is easily identified when the basal layer of any field is sharply contained. In figure 51 C, a gland on the right is filled with the surface carcinoma. A two-pronged invasive fork projects upward from the lower left and, instead of entering an area of loosened stroma, it dissects the stroma. Nearby on the left one can see irregular splinters of a few invading carcinoma cells. In other cases plexiform cords of undifferentiated cells invade the stroma, although within them differentiated pseudopearls may develop.

A third pattern of invasion is not at first glance readily discriminated from a cluster of distended, round glands entirely filled with the surface carcinoma. Blunt, round masses arranged back to back form a sheet of somewhat deeply lying cells bounded distinctly by a thickened stroma. Several features aid recognition. The sheet of invasive carcinoma is made up like a patchwork quilt, whereas a group of glands distended by in situ carcinoma either will somewhere show their tubular pattern or will appear as discrete, round masses if cut in cross section (see Fig. 46 F). The stroma limiting even a small invasive carcinoma often is the thick, coarse collagen characteristic of carcinomas, whereas that beneath a carcinoma in situ is almost invariably normal, as Schiller[10] pointed out. Finally, the epithelial cells themselves often differ from the cells in the surrounding in situ field, for it is not precisely true that the invasive portion of a

carcinoma is indistinguishable from the non invasive portion The invasive part usually is somewhat better differentiated, the surface part, more anaplastic In the invasive part the cells have more cytoplasm and tend to be somewhat larger and more variable the nuclei are somewhat more vesicular and the nucleoli are larger We have just noted that this is often the case with the earliest invasive buds as well as with the more advanced invasive growths Perhaps this is the reason that Nieburgs and Pund[9] have observed more variability among cells exfoliated from invasive carcinoma than among those from pre invasive carcinoma

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It has already been mentioned that endocervical glands filled with surface carcinoma must be recognized and distinguished from genuinely invasive carcinoma No matter how cut they are usually round regular masses sharply

* See Novak[23] Figs 4 and 5

bounded by the limiting stroma except in the presence of undue inflammation As the neighboring branches of a gland become filled and distended with the proliferating epithelial cells the intervening connective tissue is compressed into narrow stromal septa As illustrated in figure 46 F masses of intraglandular surface carcinoma may appear to be isolated deep in the stroma but this appearance results from vagary of sectioning, the branching glands connect with the topographical surface The cells filling or almost filling the glands display the cytologic atypicalities of those in the surface field elsewhere Lesions derived from endocervical reserve cell hyperplasia and metaplasia in the canal usually are poorly differentiated (Fig 46 E) In my opinion, fields occupying glands frequently arise from intrinsic reserve cell hyperplasia and metaplasia However, many authors speak of ' invasion ' of the glands, likening the process to the extension of regenerating squamous epithelium into the glands of a transformation zone Although tactile extension of a portio surface carcinoma into glands may occur ' extension" describes the process better than ' invasion' because normal tissue boundaries are not violated To construe involvement of glands alone as evidence of invasion results in labeling many cases of in situ carcinoma invasive carcinoma For example in Galvin and TeLinde s[35] series of 75 cases found by biopsy to be intraepithelial carcinoma 55 were later called invasive after pathologic study of the hysterectomy specimen It would be better to speak of extension into glands or ' involvement of glands and to retain the term ' invasive carcinoma ' strictly for lesions that actually invade the stroma The confusion which otherwise may result is exemplified in the published discussion of Galvin and TeLinde s[3] paper, in which the ' modified Wertheim hysterectomy proposed by the authors was criticised as inadequate treatment for the 70 per cent of their cases in which they discovered invasion' on study of the entire cervix after operation In truth their operation was clearly adequate because they did not describe or illustrate invasion of tissue spaces, the only avenue to local destructiveness and to lymphatic and vascular permeation The limited sense in which these authors use the term invasion has been clarified[31 6] Younge *et al*[138] consider involvement of glands a further developed stage of in situ carcinoma At least the surface covered is greatly augmented by glandular involvement and hence the area from which invasion may develop is greater Serial sections reveal invasive buds to arise alike from glandular and topographical surfaces

Whenever genuine invasion is demonstrated the growth is an infiltrative *squamous* cell carcinoma Perhaps all carcinomas do begin in the basal cells and some invasive cervical carcinomas are undifferentiated However these considerations and the long clinically latent period should not prompt comparison of in situ carcinoma of the cervix with the more benign so called basal cell carcinoma of the skin Such tumors also arise from the basal cells but they are well differentiated in the direction of the adnexal structures They arise in the skin from *distinctive often* multicentric fields but analogous tumors are rarely if ever found in the cervix probably because it normally does not develop dermal appendages

Statistical Evidence — It would seem there is now adequate anatomical evidence that invasive cervical carcinoma may arise in a previously established in situ carcinoma An important question still unanswered is How often does this occur? Too few cases of carcinoma in situ have been followed undisturbed to give an answer and as already noted it is difficult in those cases to be certain that

invasive carcinoma actually arose from a lesion which had been only in situ carcinoma One then wonders whether statistical analysis of the frequency with which in situ and invasive cervical carcinoma occur can answer the question, if a developmental relationship between the two lesions is granted

Some have thought data already available on the frequency with which the two are found indicate that carcinoma in situ almost invariably becomes invasive

In certain hospital series[50, 63, 111, 135, 138] the frequency of carcinoma in situ among female patients is about equal to the frequency of invasive carcinoma among female patients taking the frequency of invasive carcinoma of the cervix to be 1 6 per cent * It might at first thought appear that an equal frequency of the two lesions in such groups of women supports the idea that carcinoma in situ always becomes invasive However there are two main reasons why direct comparison of the relative frequency of in situ and invasive carcinoma among patients admitted to a hospital or seen in an out patient clinic actually permits no conclusions concerning the percentage of cases of in situ carcinoma that in time would become invasive The first reason is that a hospital or clinic population does not represent an unselected sample of the general female population and with regard to instances of in situ and invasive carcinoma the selection would be dissimilar The second reason is that the relative frequency of finding each type of lesion cannot be treated as the incidence rate of each

Sampling errors arise from the selected composition of a hospital population Signs and symptoms of carcinoma in situ are either absent or so trivial compared with those of invasive carcinoma that admission visits and referral of patients probably are heavily weighted in favor of invasive carcinoma It is fair to suppose that most cases of invasive carcinoma developing in a given general female population will at some time in their course appear in the hospital serving that general population Furthermore if the hospital in question provides above average gynecological consultants and radiological therapists the hospital population will be further weighted in favor of cases of invasive carcinoma because it will attract from a distance patients in whom cervical carcinoma has already been diagnosed or suspected On the other hand, a hospital or clinic population will be less likely to select cases of carcinoma in situ from the general female population Because irregular or post coital bleeding sometimes accompanies carcinoma in situ, there will be a certain degree of selection but less than in cases of invasive carcinoma In addition to selecting symptomatic over asymptomatic diseases from the general population, a hospital population may be unrepresentative of the general population in another way This is in its age distribution As reference to figure 49 shows the age distributions of cases of in situ and invasive carcinoma differ therefore the age distribution of the hospital or clinic population and its relationship to the age distribution of the general population must be known if data derived from hospital populations are to be meaningful For the reasons just given it seems apparent that comparison of the relative frequency of in situ carcinoma and invasive carcinoma among hospital and clinic populations does not answer the question and may confuse the problem when equal relative frequencies turn up fortuitously

Even if one should neglect for the moment the errors in sampling that arise from the study of hospital and clinic populations there remain certain mistakes

* This commonly quoted figure is taken from Meigs[75] who specified 1 6 per cent of married women over thirty years of age admitted to the hospital

in interpretation that have come from regarding relative frequencies as though they were incidence rates * These mistaken interpretations would be no less in correct if they were made about data gathered from populations truly representa tive of the general female population and hence, free of sampling errors In order to conclude that statistical analysis of a study of living women is consistent with the idea that all cases of in situ carcinoma would become cases of invasive car cinoma, it must be demonstrated that carcinoma in situ and invasive carcinoma *occur* that is arise at the same *rate* Let us see if this has been demonstrated by the studies cited previously Of all women studied the percentage found to have carcinoma in situ is usually designated as the incidence ' of carcinoma in situ within the population studied Similarly, the percentage found to have invasive carcinoma is usually designated as the ' incidence ' of invasive carcinoma within the population studied When each member of a given population has been ex amined one time the results of the examinations carried out permit a census of cases of both kinds to be made From such a census an ' incidence *rate* ' cannot be derived because the frequency of each lesion has been determined only on a space base ' and not, in addition on a time base ** If one wished to use one year as the time base the annual incidence rate of cervical carcinoma in situ in a given female population would be the number of new cases that developed during the period of one year within the population per some convenient unit of the population as per 100 per 1000 or per 10 000 women Similarly the annual incidence rate of invasive cervical carcinoma in a given female population would be the number of new cases of invasive cervical carcinoma that developed during the period of one year within that population per unit of population chosen These two incidence rates could be compared to determine whether or not the two con ditions arise with equal frequency Theoretically it would be possible to deter mine the annual incidence rate of a condition like measles by a single census because the census taker could expect a reasonable answer to his question Has there been a case of measles in the household the past year? However the annual incidence rates of carcinoma in situ and invasive cervical carcinoma could not be determined by the method of a single census because the patient would not be certain whether or not she suffered from the condition in question and more important for this discussion in cases where a lesion was demonstrated no one could know when it arose Therefore a single examination of a given female population should disclose ideally all cases of in situ and invasive carcinoma but could not disclose when they arose From the data obtained what has been termed the *prevalence* [4] could be computed The prevalence of a condition in a given population is simply the total number of existing cases per some chosen unit of population Since the average durations of both in situ and invasive carcinoma are greater than one year the prevalence of each would exceed the annual incidence rate of each

In comparing the prevalences of two conditions two sets of variables must be taken into account These are the incidence rates of both conditions and the average durations of both conditions For example if we accept the hypothesis that every case of in situ carcinoma becomes invasive and that every case of invasive carcinoma arises from an in situ carcinoma the incidence rates of the

I am indebted to Dr John F Dunn Jr Chief Field Investigation Section Cancer Control Branch National Cancer Institute Bethesda 14 Maryland for a discussion of this problem

See Pearl[34] for a discussion of rates

two would be equal neglecting deaths before there was time for invasion to occur In this case the ratio prevalence of in situ carcinoma to prevalence of invasive carcinoma would equal the ratio average duration of in situ carcinoma to average duration of invasive carcinoma The ratio of prevalences would be unity only if the average durations were equal However, it has already been noted that the average duration of the in situ stage is approximately 5 times longer than the average duration of untreated invasive carcinoma When the average duration of each stage is taken into account it is clear that carcinoma in situ would be 5 times more prevalent than invasive cervical carcinoma if the stated hypothesis should be valid (If treatment were taken into account, both average duration and prevalence of invasive carcinoma would be increased and the ratio, 5 to 1, correspondingly decreased)

It is apparent that the frequency of finding in situ and invasive carcinoma in an unselected female population by means of a single examination measures the prevalence of each and not the incidence rate of each Returning again to a discussion of the data on the frequency of finding in situ and invasive cervical carcinoma in hospital and clinic populations it should be clear that the relative frequency of finding such lesions is of only limited value for estimating rates applicable to the general female population Insofar as in situ carcinomas are identified in asymptomatic women the frequency of finding this lesion in such groups will provide approximations of the age specific prevalence rates in the general population The same would not be true for the frequency of finding invasive cervical carcinoma since these cases are frequently symptomatic and as already noted symptomatic individuals from a community are concentrated in clinic and hospital populations Unless the proportion of all women hospitalized and the proportion of all women with invasive carcinoma hospitalized are known, the data from hospital populations cannot be related to the general population Under optimal circumstances the number of cases of cervical carcinoma seen in hospitals can be used to obtain an approximation of the incidence rate of symptomatic cervical carcinoma in the general female population For example in Denmark[13] the population is small and relatively stable and there are few hospital centers for the treatment of cervical carcinoma With the cooperation of these centers the Danish National Cancer Registry is informed about cases of cervical carcinoma appearing for treatment Under such conditions it would be possible to determine the number of *new* cases of symptomatic cervical carcinoma appearing each year in the general female population assuming that most symptomatic women seek medical attention In the United States however it is doubtful that the relative frequency of finding invasive cervical carcinoma among hospital patients can be used to measure either the prevalence or the incidence rate in the general female population

If one were to determine from prevalence data alone the frequency with which in situ carcinoma becomes invasive the following method might theoretically be used A census would have to be made to determine the prevalence of in situ cervical carcinoma among a given female population representative of the general female population in age race social and marital composition Then an hypothetical population of similar composition would have to be followed on standard life tables for approximately twelve years (taking twelve years to be the combined average duration of the in situ stage and the untreated invasive stage) in order to take into account deaths from causes other than cervical carcinoma Next, the

in interpretation that have come from regarding relative frequencies as though they were incidence rates.* These mistaken interpretations would be no less incorrect if they were made about data gathered from populations truly representative of the general female population and, hence, free of sampling errors. In order to conclude that statistical analysis of a study of living women is consistent with the idea that all cases of in situ carcinoma would become cases of invasive carcinoma, it must be demonstrated that carcinoma in situ and invasive carcinoma *occur*, that is, arise at the same *rate*. Let us see if this has been demonstrated by the studies cited previously. Of all women studied the percentage found to have carcinoma in situ is usually designated as the "incidence" of carcinoma in situ within the population studied. Similarly, the percentage found to have invasive carcinoma is usually designated as the "incidence" of invasive carcinoma within the population studied. When each member of a given population has been examined one time, the results of the examinations carried out permit a census of cases of both kinds to be made. From such a census an "incidence *rate*" cannot be derived because the frequency of each lesion has been determined only on a "space base" and not, in addition, on a "time base."** If one wished to use one year as the time base, the annual incidence rate of cervical carcinoma in situ in a given female population would be the number of new cases that developed during the period of one year within the population per some convenient unit of the population, as per 100, per 1000, or per 10,000 women. Similarly, the annual incidence rate of invasive cervical carcinoma in a given female population would be the number of new cases of invasive cervical carcinoma that developed during the period of one year within that population per unit of population chosen. These two incidence rates could be compared to determine whether or not the two conditions arise with equal frequency. Theoretically, it would be possible to determine the annual incidence rate of a condition like measles by a single census because the census taker could expect a reasonable answer to his question, "Has there been a case of measles in the household the past year?" However, the annual incidence rates of carcinoma in situ and invasive cervical carcinoma could not be determined by the method of a single census because the patient would not be certain whether or not she suffered from the condition in question, and, more important for this discussion, in cases where a lesion was demonstrated no one could know when it arose. Therefore, a single examination of a given female population should disclose, ideally, all cases of in situ and invasive carcinoma but could not disclose when they arose. From the data obtained what has been termed the *prevalence*[1] could be computed. The prevalence of a condition in a given population is simply the total number of existing cases per some chosen unit of population. Since the average durations of both in situ and invasive carcinoma are greater than one year, the prevalence of each would exceed the annual incidence rate of each.

In comparing the prevalences of two conditions, two sets of variables must be taken into account. These are the incidence rates of both conditions and the average durations of both conditions. For example, if we accept the hypothesis that every case of in situ carcinoma becomes invasive and that every case of invasive carcinoma arises from an in situ carcinoma, the incidence rates of the

* I am indebted to Dr. John E. Dunn, Jr., Chief, Field Investigation Section, Cancer Control Branch, National Cancer Institute, Bethesda 14, Maryland, for a discussion of this problem.

** See Pearl for a discussion of rates.

expected deaths from cervical carcinoma among such an hypothetical population during the twelve year period would have to be calculated from accurate, age specific cancer mortality statistics and to this number added the estimated number of cases of cervical carcinoma in situ dying before invasion could develop and the estimated number of cases of cervical carcinoma cured by treatment during the same period of time. Finally, the adjusted total number of cases of invasive cervical carcinoma thus derived could be compared with the total number of cases of in situ carcinoma originally found in order to determine the approximate frequency with which in situ carcinoma would become invasive, again granting that there is a developmental relationship between the two lesions. The practical objection to the procedure is that at least in this country mortality statistics are notoriously inaccurate concerning the cause of death.*

Dunn[24] has suggested a method whereby the prevalence of in situ and invasive cervical carcinoma and the incidence rate of in situ carcinoma could be obtained from successive examinations of the same population. A stable female population representing a sample of the general female population would be surveyed to determine the age specific prevalence of in situ carcinoma and the age specific prevalence of invasive carcinoma of the cervix. This would be accomplished by a combination of exfoliative cytological biopsy and clinical techniques. Dr Dunn has suggested a number of uses to which these data might be put but for the purpose of this discussion major interest attaches to the results of a second survey. One year later the *same* population would again be examined to determine the number of *new* cases of in situ carcinoma and of invasive carcinoma that had arisen during the intervening year. From the new cases the annual age specific incidence rates of carcinoma in situ could be calculated. If the study were done with a population for which the annual incidence rate of symptomatic cervical carcinoma were already known the two incidence rates could be compared to determine the approximate proportion of in situ carcinomas that would become invasive. Other data derived would depend on whether or not cases of carcinoma in situ found in the first survey were treated. This general method is sound and properly applied should in a short period of time give reliable information concerning the approximate frequency with which cervical in situ carcinomas become invasive.

It can be concluded that statistical analysis of the comparative frequency of finding in situ and invasive cervical carcinoma among living women has offered thus far no information concerning the comparative rates at which the two arise. Therefore such studies offer no basis for estimating the frequency with which in situ carcinoma develops into invasive carcinoma.

The Evidence That Invasive Carcinoma May Not Develop in Fields of Carcinoma in Situ and in Atypical Cervical Epithelium —*Case Studies* —For every case of carcinoma in situ reported to progress clinically to invasive carcinoma there are many reported to remain well but in the great majority of the latter group the entire surface field was probably removed by some form of treatment. The failure of carcinoma in situ to progress to invasive carcinoma after conization trachelectomy cervical amputation cauterization or irradiation is not valid evidence that carcinoma in situ does not necessarily become invasive. Wespi[131] has followed a few cases in which carcinoma in situ did not develop into

* For a discussion of mortality statistics and cause of death see Pearl[9] and for a discussion of inaccuracy in the diagnosis of malignant neoplasm see Willis[134] and Wells.[130]

TABLE VIII.—CASES OF CARCINOMA IN SITU NOT PROGRESSING CLINICALLY TO INVASIVE CARCINOMA†

Author	*Case No.*	*Carcinoma in situ present at age*	*Carcinoma in situ demonstrated by*	*Status last reported*	*Duration of Follow-up*	*Manipulation, etc., in interim*
Diddle *et al.*[21]	8	24	Biopsy	Normal on pathological examination (Hysterectomy)	4 yrs.	Nothing
Galvin[?]	10	47	Biopsy	Carcinoma in situ, questionable invasion (Conization)	6 8/12 yrs.	Nothing
Knight[4]	4	53	Curettage	No abnormalities on physical examination	5 yrs.	Estrogens, douches
	5	45	Amputation	Normal physical findings	About 6 yrs.	Two biopsies
Stevenson and Scipiades[?]	11	27	Biopsy	Alive and well	6 yrs.	Nothing
Wespi[131]	48	42	Biopsy	Atrophic epithelium (Colposcopy and biopsy)	13 2/12 yrs.	Nothing
	49	29	Biopsy	Inflamed transformation zone (Biopsy)	16 1/12 yrs.	One Biopsy
	51	30	Biopsy	Transformation zone, no atypical epithelium (Biopsy)	3 3/12 yrs.*	Biopsies
	52	23	Biopsy	No atypical epithelium (Biopsy)	1 6/12 yrs.	Biopsy

† Cases with multiple biopsies, conization, trachelectomy, cervical amputation, cauterization or irradiation not included.
First after 5 yrs. revealed carcinoma in situ still present.
Wespi[31] also accepts Scipiades and Stevenson's[112] cases 2, 3, 4, 6 and 7 (Stevenson and Scipiades's[125] cases 6, 7, 9, 8 and 4 respectively) but in their American report[25] the authors indicated that the entire lesion probably was removed in cases 7, 9 and 4 and cauterization was done in cases 6 and 8.
* Short follow-up period but colposcopic and histologic evidence of regression.

monal changes in the cervix There are no alterations of the cervical epithelium peculiar to pregnancy The squamous epithelium is thickened both the glycogen bearing rete malpighii and the psuedo keratinized surface More pertinent to this discussion is an increased incidence of basal cell hyperactivity The thickness of the basal zone may increase from the usual 2 to 4 cells to 8 or more Nuclear variability, frequency of mitosis and incidence of multiple nucleoli are increased in pregnancy Large nuclei may survive in the upper rete malpighii where normally they become shrunken and somewhat pyknotic Fluhmann[30] reported the development during pregnancy of endocervical ectopia (so called erosion) in about half of 119 private patients The lesion was present as frequently in primigravidas as in multigravidas emphasizing that not the trauma of delivery but pregnancy, itself causes the lesion The epithelial hyperplasia and regeneration in the transformation zone of an ectopia and their relationship to the genesis of carcinoma in situ have already been discussed in detail Some years ago Hofbauer[59] described basal or reserve cell hyperplasia and squamous metaplasia in pregnancy Even though this is a common lesion in the non pregnant uterus, it seems probable that it is often established because of the prolonged estrogenic stimulus of pregnancy From a careful histologic study of a relatively small group of pregnant cervices 22 in all Danforth has illustrated 4 examples* of what I would interpret as metaplasia with cytologic atypicalities Additional studies of this kind are needed to determine the frequency of such atypical metaplastic epithelium in cervices from pregnant compared with non pregnant uteri

If pregnancy and its altered hormonal state stimulate epithelial hyperplasia and also probably produce lesser degrees of cytologic abnormality such as ' basal cell hyperactivity and ' metaplasia with atypicality in the cervix it is reasonable to conjecture that carcinoma in situ might occur unexpectedly often in pregnancy At just this point available data are inadequate In a histologic study of 37 pregnant uteri Pund *et al* [100] reported 4 preinvasive cervical carcinomas an incidence of 11 per cent and in addition one ' covert invasive carcinoma This report was made in 1948 Now the number of cervices from pregnant uteri studied by serial blocks in Pund s laboratory totals 107 In this series 9 pre invasive carcinomas have been found an incidence of 8 4 per cent [103] This obviously high frequency contrasts with none found in 23 whole specimens (17 autopsy 4 hysterectomy, and 2 amputation) studied by Glass and Rosenthal [39] Excluding a group of abnormal appearing cervices studied one finds that Carrow and Greene[10] discovered 2 carcinomas in situ among 64 normal appearing cervices (10 hysterectomy 54 biopsy specimens) an incidence of about 3 per cent In a series of cervical biopsies of 286 pregnant patients Epperson *et al* [27] found 2 intraepithelial carcinomas an incidence of less than 1 per cent It is apparent that the frequency of cervical in situ carcinoma in pregnancy remains to be determined and that even an estimation would be hazardous

There is rather general opinion that carcinoma in situ discovered during pregnancy may regress afterward For this reason the diagnosis of in situ carcinoma during pregnancy is taken with reservation even by some who consider the lesion inevitably invasive As already noted the question of how often in situ carcinoma becomes invasive is not settled in the non pregnant or the pregnant state Moreover there is as yet little basis for discussion of the lesion s reversibility following

* See Danforth[20] Figs 11 12 13 and 14

invasive carcinoma even though the initial lesion was disturbed only to take the biopsy sample. It is conceivable that the entire field was removed at the time of diagnosis, but he thought it unlikely. Cases of this kind are summarized in Table VIII. One may object that the findings are not conclusive that insufficient time may have elapsed to permit invasion if the latent period was unusually long. The argument is not readily refuted, except in cases where objective evidence of the lesion disappeared. The list of non progressive cases in Table VIII is shorter than the list of progressive cases in Table VII, p. 230. This is, however, no indication of the proportion of cases that belongs in each category. Many of the cases in Table VII were discovered by reviewing former biopsies of patients presenting with the complaints of a fully developed malignant tumor. Obviously, this weights the data since in non progressive cases the patients have no reason to return to a gynecologist. Only by tracing all patients in a group with a biopsy of carcinoma in situ can some measure of the actual proportions be learned. A recent review[65] refers to a study now in progress at the Radiumhemmet in which more than 40 patients with in situ carcinoma of the cervix are deliberately being followed without any kind of treatment. Such a study is long overdue. Great patience will be required to leave these lesions undisturbed for years but through a painstaking investigation of this kind the likelihood that invasion will develop in fields of in situ carcinoma of the cervix can be accurately determined.

Wespi[131] presents quite sound evidence that neither surface nor invasive carcinoma inevitably ensues if only the lesser degrees of cytologic atypism outlined in figure 49 are present. Thirteen cases of "irregular epithelium" regressed judged by subsequent histologic examination and 3 more as determined by colposcopy; 2 cases progressed to "increasingly atypical epithelium"; 4 remained stationary a few months. Wespi[131] also cites 15 cases reported by Scipiades and Stevenson[117] in which "hyperactive epithelium" did not progress to carcinoma. Still lesser degrees of epithelial abnormality are found so frequently that no one would contend they progress obligatorily to carcinoma.

Statistical Evidence.—Whereas some statistical studies might indicate that in general hospital populations carcinoma in situ is found at a frequency equal to that of invasive carcinoma, others indicate that the frequency is higher. In histologic studies of uteri removed for non cervical diseases unsuspected cervical carcinoma in situ has been found in as many as 3.9[99] and 3.5 per cent[6] of cases. However, Pund *et al.*[100] think that the frequency of 3.9 per cent in their series is not unexpectedly high if allowances are made for selection of cases in which total hysterectomies were done and for loss of cases due to deaths from other causes during the calculated latent period of twelve years preceding invasion. The difficulties in interpreting data of this kind have already been considered. It is not possible to relate these frequencies to the frequency with which carcinoma in situ progresses to invasion.

Carcinoma in Situ in Pregnancy.—It has been suggested that carcinoma in situ of the cervix is found unusually often in pregnant uteri and that the lesion may regress after parturition. There have been only a few investigations specifically directed toward verifying these two beliefs, and even tentative conclusions are very likely premature. The studies[10, 0, 7, 30, 39, 59, 85, 119] which have been made thus far indicate that there is increased cellular activity and hyperplasia of the cervical epithelium during pregnancy. Novak has suggested that definition of these alterations in pregnancy is part of the problem in defining the cyclic hor

this kind prove that a field of surface carcinoma does not always precede invasive carcinoma, they are uncommon exceptions to the rule Cases of both kinds seem to be so uncommon that they would not vitiate scientific statistical studies to determine the frequency with which in situ carcinoma becomes invasive

Criteria of Malignancy Other Than Invasiveness —In the foregoing discussion emphasis has been laid on the fact that carcinogenesis is a process There are legitimate differences in opinion as to the stage in the process that should be labeled "malignant for this is a relative term no matter how absolute it sounds To many who consider cytologic and nuclear atypism indication of a fundamental disturbance in cellular function and perhaps genetic constitution, invasion is only an incident in the abnormal growth process In discussing the origin of carcinomas of the breast from intraductal epithelial proliferation, Cheatle and Cutler[12] noted that the abnormal cells in the ducts often look like those that are invading They pointed out that in a microscopic invasive focus, cells can sometimes be found half inside and half outside a duct Remarking that the external halves of these cells cannot be described as precancerous they contended that such cells half inside and half outside normal boundaries demonstrate that the cells inside the duct are as malignant as those invading the tissues outside it However one who holds that malignancy is determined by abnormal relationships of tissues would consider that the characteristics of a cell half inside and half outside the duct are not so important as the getting it there Even so we have already seen that in the cervix the morphologic character of the invading cells often is different from that of their non invasive neighbors when studied by ordinary histologic techniques This observation finds support in data from certain refined cytologic studies of experimental carcinogenesis Cowdry and Paletta[15] compared the pre invasive, hyperplastic mouse epidermis induced by methylcholanthrene with the later carcinomatous epithelium The malignant cells they found, were smaller than *the hyperplastic spinous cells but larger than the hyperplastic basal cells* the nucleocytoplasmic ratio larger in carcinoma cells than in spinous cells but about equal with basal cells the nucleoli, larger and more numerous in malignant cells The details of the differences they noted are not identical to the differences we have pointed out between invasive and non invasive cells of some cervical carcinomas Although they concluded that no single criterion differentiated the malignant cell from the hyperplastic cell the fact remains that the cells of an invading neoplasm are often not identical with the cells in the non invasive antecedent field of origin

Carruthers[11] has recently reviewed the chemical alterations that have been demonstrated during the experimental transformation of mouse epidermis to squamous cell carcinoma In a short space the various alterations in minerals vitamins lipids and amino acids cannot be entered into In general however there is a sharp shift in proportions of a number of substances early in the hyperplastic stage and another shift in the malignant phase For technical reasons none of the histochemical alterations has been pin pointed at a malignant focus Study of the earliest microscopic foci of invasion would be difficult because they are found only by chance

The ability of tumor cells to proliferate after heterologous transplantation has been offered as a biological test of malignancy In recent years Greene[16–18] has successfully transplanted a number of human and animal tumors into the anterior chamber of the eye of a heterologous species Without entering into the dis

pregnancy. Younge *et al*,[133] report 2 cases regressing to "anaplasia" after pregnancy. Epperson *et al*,[27] report 5 cases discovered during pregnancy in all of which the in situ carcinoma was no longer found in postpartum biopsies. Unfortunately, the last study loses much of its significance because of repeated biopsies and even a conization in one case. Conclusions about the potentiality of a lesion can hardly be based on the outcome following repeated removal of samples for biopsy.

Cervical Carcinoma and Two Special Groups of Women.—There is opportunity in two selected populations for statistical studies which might possibly give evidence about the reversibility of carcinoma in situ. It is known that the incidence of genuine cervical carcinoma in Jewish women is astonishingly low. Of importance in the whole problem of the pathogenesis of the tumor is the provocative report by Gagnon[34] that squamous carcinoma of the cervix is practically nonexistent in nuns. Gagnon also noted that inflammatory erosions in this select group are rare. It would be of great value to know whether the prevalence of carcinoma in situ parallels the prevalence of invasive carcinoma in these two groups of women. If the prevalence should be no different from that in the general female population, it would be evident that, at least in these two groups, the lesion often is non-progressive. The results of a current study[1 6] on the occurrence of the in situ lesion in Jewish women will be awaited with interest.

The Origin of Invasive Squamous Carcinoma of the Cervix Unrelated to Fields of Carcinoma in Situ.—For biological, statistical and practical reasons it is important to know whether invasive cervical carcinomas always develop from fields of in situ carcinoma. Wespi's colposcopic and pathologic studies[131] of the histogenesis of the neoplasm indicate that it usually does arise in this way; the histologic observations of Pund and Auerbach[99] support this concept; my histologic studies of early carcinomas agree with these observations. Schiller's studies[110] of twenty five years ago suggest the same conclusion. It is, of course, more difficult to determine the origin of advanced invasive growths, but the observations of Cullen[17] and of Schottlaender and Kermauner[116] are consistent with this same viewpoint. Even in the presence of obvious tumors such as they studied, a portion of the surface field not yet altered by invasion can often be found about the margin of the invasive area. Moreover, the development of a shower of many invasive foci in a large field of in situ carcinoma very likely accounts for the considerable number of extensively invasive neoplasms found in the presence of clinical signs of only a few weeks duration. But there are a few observations demonstrating other anatomical patterns in early invasive carcinomas. Some years after publishing his monumental volume on *Cancer of the Uterus*,[17] Cullen reported the chance finding of a tumor, calling it "the earliest case of squamous cell carcinoma of the cervix that I have ever seen."[18] This was a polypoid growth 9×3 millimeters at the upper end of the endocervical canal. The photomicrographs show a focus of carcinomatous invasion, although elsewhere a field of metaplasia with atypicality is present in the endocervical mucosa of the polypoid projections. One rarely happens upon a small carcinoma much like many epidermal acanthomas that arise in a limited field. In these instances it is possible that all or most of a small field became invasive before the lesion was discovered.[56] Observations of another kind do not admit this interpretation. Sometimes invasive sprouts may penetrate directly from the basal layer of a normal appearing or a hyperplastic surface epithelium.[131] Although instances of

will for some time to come make his greatest contribution in apprehending the early Stage I squamous carcinomas and promptly instituting treatment

Some find the Schiller test useful in detecting carcinoma in situ occupying the face of the cervix others do not Presumably it is useful to examiners who continue to apply it and there is little point in debating how useful it is Anyone seriously interested must try it for himself in his own particular situation After the iodine solution is applied the areas of normal glycogen containing squamous epithelium are colored deep brown Since the surface carcinomas of the portio usually lack cytoplasmic glycogen they are not stained nor are other nonglycogenated surfaces—abrasions keratotic squamous epithelium and endocervical ectopias The exact nature of the abnormal areas encountered must be determined therefore by a combination of gross study and biopsy It would seem that the test might be quite useful in assessing the extension of the field onto the outer rim of the cervix once the presence of a carcinoma in situ is confirmed by biopsy

Some time ago Hinselmann devised the colposcope and it has been enthusiastically employed by several European investigators In principle the instrument is like a binocular dissecting microscope designed for examining the well illuminated cervix in the living patient Every method of preparing material for magnification and every degree of magnification create new worlds requiring interpretation and the image with the colposcope is not simply an enlarged version of the unaided eyes view of the cervix nor is it a low power magnification of a histologic section Therefore only investigators who have had a wide experience with the technique can fairly evaluate it A discussion of colposcopy and its role in cancer detection can be found in Wespi s monograph [131] It is important to realize that he personally has compared gross examination colposcopic picture and corresponding histologic sections It seems unlikely that the advantages and the limitations of the method can be learned in any other way It is emphasized that this technique is not one for examining individual cells in vivo The picture depends on features such as thickness and opacity of the epithelium and pattern of underlying blood vessels

The exfoliative cytologic method of Papanicolaou for the study of individual cells has come to assume a prominent place in cancer detection Application of the method to this problem rests on recognition of the cytologic atypicalities that usually distinguish cells from neoplasms from other cells Since the cytologic characteristics of carcinoma in situ are similar to those of invasive carcinoma abnormal exfoliated cells from these surface fields can be recognized in vaginal and cervical smears A separate section of this chapter is devoted to the subject pp 261–277

The sponge biopsy [28] is another technique for the study of isolated cells A Gelfoam sponge is rubbed over the face of the cervix exfoliated cells being trapped in the meshes of the Gelfoam The entire sponge is then placed in a routine fixative such as 10 per cent formalin and dehydrated and infiltrated by usual histologic techniques Sections are cut on the microtome from the side of the block that had been passed over the cervix Ordinary staining techniques used for paraffin sections can be applied If this technique were combined with a simple method of sampling the endocervical canal it should be quite useful in detection surveys because the specimen can be processed in any pathological

state in pregnancy, or the trauma at delivery or neither, is directly responsible is unsettled, but the development of endocervical ectopia during pregnancy is surely significant in the pathogenesis of this neoplasm Murphy and Herbut[83] noted that inflammation in the squamo columnar junction area is 3 times more common in multigravidas than in primiparas and speculated that a local concen tration of estrogen in this inflamed stroma may be etiologically important for carcinogenesis The possible importance of hormonal stimuli is further suggested by the experimental production of cervical and vaginal squamous carcinomas in mice given estrogen for a relatively long time [94] Whether there is in the human being significant correlation between cervical carcinoma and hyperestrogenism is still a disputed point but the studies of Ayre[4] on the etiologic role of hyperestro genism and thiamin deficiency deserve amplification and confirmation It should be noted that Nieburgs[88] has suggested, on the other hand, that a relative defi ciency of estrogen is associated with cervical carcinoma One cannot avoid think ing that some additional stimulus or stimuli bring about carcinomatous changes in the fields of hyperplastic epithelium found so frequently in the inflamed trans formation zone It is conceivable that the recurrent epithelial hyperplasia is the provocative stage following some as yet unrecognized initiating stage of cervical carcinoma in the sense of Berenblum and Shubik's[6, 7, 11, 177, 123] animal experiments These investigators found that repeated application of a non carcinogenic irritant such as croton oil was capable of provoking squamous carcinoma in skin previously treated by a single initiating application of a carcinogen given in a dose insuffi cient to induce carcinoma or even recognizable hyperplasia An hypothesis of this sort is at least in keeping with the anatomical development of squamous carcinoma of the human cervix

IMPLICATIONS FOR CLINICO-PATHOLOGIC MANAGE-MENT OF CARCINOMA IN SITU OF THE CERVIX UTERI

Detection of Carcinoma in Situ —A carcinoma in situ may be present in a cervix that appears grossly normal and no sign or characteristic history may presage it Despite this a hopeless attitude toward the discovery of many of the cases is unnecessarily pessimistic because up to 80 per cent will accompany an endocervical ectopia or pseudoerosion and from one fourth to one half will have a history of some kind of abnormal bleeding Post coital spotting is a suspicious sign One cannot reasonably expect leukorrhea and a necrotic mass of new tissue with lesions of this kind The signs and symptoms that may be present are of course nonspecific and most ectopias will prove entirely benign The expected clinical incidence based on present reports can be no more than 1 5 to about 4 per cent The examiner is therefore faced with demonstrating the absence of many in situ carcinomas for every one detected Many patients will never consult a physician during the in situ tage because of the trivial nature of the complaints and only widespread prophylactic surveys of apparently normal women would ferret out the cases without signs or symptoms Nevertheless careful speculum examination of the cervix during physical examinations is a simple first step in cancer detection And too as Novak[9] has pointed out the current interest in carcinoma in situ should not inadvertently slacken efforts to detect unadvanced overt carcinomas In the over all fight against cervical cancer the practitioner

9 and negative in 1 The last case was detected by smears and the diagnosis was confirmed by histologic study of the resected uterus A more satisfactory specimen is obtained by the operative removal of an endocervical cone as Martzloff[73] has illustrated If the latter procedure is done the canal should not be disturbed by previous curettage

The great advantage of the cone biopsy is that it permits mapping the lesion and systematic search for invasion The cone should be removed with a scalpel to avoid the serious artefact produced by any kind of electrical cutting instrument The base of the cone should be a ring encircling the transformation zone and the height of the cone should include about 2 centimeters of the canal I prefer to have the entire specimen fixed in one piece The anterior and posterior lips should be clearly specified After fixation, the cone is cut into thin—about 2 millimeters—sagittal blocks consecutively numbered according to a diagram The first sections are taken from each paraffin block and if provisional study of these sections indicates the need selected blocks are cut serially For research purposes I have systematically saved every fifth section for study, but there are practical limitations to be recognized If sections are cut at 6 micra thickness a sample is obtained about every one tenth of a millimeter if only every fifteenth section is retained Anything short of complete serial sections is an admitted compromise with practicality, and every laboratory will naturally work out the details best suited to its facilities and requirements

There are obvious difficulties in cervical conization for biopsy It is not an out patient procedure and therefore the demands on physician and clinic and expense to the patient are increased Post operative hemorrhage is sometimes troublesome It seems to be general experience that only some surgeons are adept enough to avoid post operative strictures and they, only if the patient will return for regular dilatations Many of these objections apparently have been overcome by Gusberg[49 50] who has described and illustrated a simple bi valved instrument for obtaining a diagnostic cone He described a relatively minor procedure which causes little bleeding and few complications The cone obtained is however small An instrument can be selected from a series of graduated sizes to match the size of the cervix but Gusberg mentions that several bites are necessary if the external os is wide and transverse Even when a scalpel is used for conization special difficulties arise if an endocervical ectopia extends far onto the face of the cervix because a Sturmdorf procedure is almost required to include all of the lesion in the specimen *In these cases, some might prefer a hysterectomy in order to* obtain the entire cervix for thorough histologic study, others might prefer a shallow flat ring excision of the cervical lips and an endocervical scraping There will certainly be cases in which proper management calls for a procedure either more or less extensive than conization because diagnostic and therapeutic procedures overlap Despite the disadvantages mentioned a cone specimen obtained in one piece allows one to map the field to determine whether contiguous parts of the field remain in the cervix and to thoroughly study the field for invasive foci These advantages are especially important since the gross appearance of the lesion is often deceptive

A reliable biopsy is possible only with a satisfactory specimen An expensive time consuming histologic study is hardly worthwhile on tissues altered by an electrical current The specimen should be removed with a sharp instrument and then the cautery may be used if it is deemed necessary or advisable The

laboratory Furthermore, the stained section should be readily interpretable by any pathologist

The Biopsy of Carcinoma in Situ —Any combination of methods may uncover the lesion, but no matter how the preliminary studies are done, identification of carcinoma in situ depends on histologic evaluation of a properly obtained specimen for biopsy Many cases are first detected by biopsy, and a number of clinics rely chiefly on this method to uncover the lesion If reasonable experience on the part of the pathologist is assumed, a diagnosis of carcinoma in situ means *one of two things* *Either the cervix contains only a carcinoma in situ or else* somewhere in the field but outside the area sampled histologically, there is also invasion at one or more sites Unless the cervix has been thoroughly examined and any grossly suspicious area taken for biopsy, an obviously invasive lesion may be missed and only the surface portion of the lesion included in the specimen This same problem arises when small invasive lesions occur in part of the field occupying the endocervical canal Pund and associates[100, 101] have called them ' covertly invasive carcinomas which may not become overt until they have undermined the portio epithelium and broken through it Furthermore, one can hardly guarantee the absence of microscopic foci of invasion like those illustrated in figure 51 short of thorough, ideally serial histologic sectioning of the entire lesion Within the bounds of practicability then, methods which stand the best chance of providing *material for an accurate evaluation must be systematically applied* No one approach can be dogmatically recommended but in order to avoid confusion the clinician and the pathologist must cooperate so that each knows what the other is doing and why he is doing it

Selection of the specimen for biopsy presents a special problem since no distinctive gross features serve as guides excluding from discussion fields containing a small, but evident invasive carcinoma Those who wish to use additional methods of gross examination may find the Schiller test and colposcopy helpful in determining the sites to be chosen Otherwise, the guiding principle is that the transformation zone and its squamous epithelial margin should be sampled Based on a study of 27 cases Foote and Stewart[31] found that about half of the cases would have been discovered in a biopsy sample from either anterior or posterior lip more than two thirds if both had been sampled and 25 of 27 if both lateral angles and both lips had been sampled Howard *et al*[6] found however that only 2 of 14 cases would have been discovered by a single full thickness block removed from the operative specimen These studies show that chance enters into the discovery of in situ carcinoma and as expected that the chances are increased with increasingly wider sampling One of the theoretical advantages of exfoliative cytologic study is that the entire cervix and canal are sampled in properly obtained smears

The detection of lesions within the canal by biopsy sampling and the verification of such lesions when they are suspected by smears present additional problems Two methods are applicable The simplest method is to obtain cervical scrapings with a curette or with a spoon in addition to the usual punch biopsies The effectiveness of this method is illustrated in Pund and Echols [10] report of 54 cases of "preinvasive" carcinoma in which both excised biopsy samples and endocervical scrapings were taken In 25 cases the surface carcinoma was found in both the biopsy sample and the endocervical scraping In 44 cases the biopsy sample was positive in 10, negative In the latter 10 cases the scraping was positive in

nosis of carcinoma in situ is established by biopsy These procedures likewise provide a specimen which can be thoroughly studied in serial sagittal blocks If invasion is found in the operative specimen, intracervical application of radium is of course, not possible but roentgen ray treatment is

Since operative treatment is effective there seems to be no reason for primary irradiation of the non invasive lesion One of the great disadvantages of irradiation is that there is no secure means to determine the nature of the lesion that is being or has been treated If because of uncertainty, every lesion must be treated as though it were already invasive the non invasive, as well as invasive, cases must be subjected to full and proved therapeutic courses and, hence to the risks of post radiational complications In addition ovarian function of young women is unnecessarily destroyed in cases of no more than in situ carcinoma Despite these considerations irradiation is used in a number of clinics [61]

There is place for considerable individualization of treatment Younge *et al* [138] for example do not hesitate to follow a young patient who may desire children citing the long average in situ period as a probable safeguard Until more is known there is good reason before instituting treatment to consider the importance of the personal and social situation when there is reasonable assurance that the lesion is only in situ carcinoma

If a number of operative specimens are thoroughly studied cases with genuine microscopic stromal invasion at the time of operation will be discovered The proportion of such cases discovered likely varies with the thoroughness of histologic study, but the relative frequency remains to be determined The solution of the problem is of obvious importance before any sensible answer can be given to the question of how radical the operative procedure should be if and when operative treatment is decided on Data presently available are too variable to be conclusive Gusberg[50] reported microscopic invasion in 5 of 10 cases investigated by considerably less than serial sections of cone biopsies he illustrated one such invasive focus distinguishing true invasion from glandular involvement In a later series[51] the same investigator found microscopic invasion in only 15 per cent of 66 cases Foote and Stewart[31] found invasion in only 1 of 28 cases Younge *et al* [138] found definite invasion in 7 of 135 cases and probable or questionable invasion in 12 additional instances Douglas and Studdiford [3] reported unsuspected invasion in 3 of 16 cases If penetration of lymphatic channels is not demonstrated in addition to stromal invasion, there is considerable question that more should be done if a hysterectomy has already been performed The decision will perhaps depend on the thoroughness of the histologic studies It is at least the pathologist s duty to make the situation clear and not to report simply squamous cell carcinoma or early squamous cell carcinoma Clarifying the situation actually means clearly explaining the problem I have found that the best explanation is a microscopic demonstration After a few demonstrations a report is readily comprehended when it states that a single microscopic focus or multiple microscopic foci of stromal invasion (like those illustrated in Fig 51) have been demonstrated in a wide field of carcinoma in situ If this kind of report does not say simply that the lesion is or is not a carcinoma it has the virtue of adhering to demonstrated fact accurately depicts the development of the pathologic process and does not err in making overly simple a problem in treatment which is, after all a problem

usual punch biopsy forceps have limitations because the specimens obtained are small and not easily oriented to permit sectioning perpendicular to the surface It would be a distinct advance if the forceps described by Younge *et al*[138] came into widespread use They enable the operator to obtain a sharply incised rectangular block which can be properly oriented for paraffin imbedding Each *specimen should at least be labeled e g , anterior, posterior, left right, canal, etc* A simple diagram of the lesion and the sites sampled is extremely useful for future reference Every cone biopsy should be oriented so that the reconstructed map can be referred to the uterus In short the aims of the diagnostic procedure should be understood alike and agreed upon by surgeon and pathologist if the patient's interests are to be properly served These aims depend at least in part on one s understanding of carcinoma in situ and point of view toward treatment

Implications for Treatment —The present uncertain state of knowledge precludes categorical principles of therapy Critical understanding of the etiology or etiologies of carcinoma in situ is wanting Why the field develops in one inflamed ectopia and not in another or indeed in a virtually normal looking cervix, remains unknown Since the conditions provoking invasion in the field are obscure there is hardly reason to suppose anyone could predict in which cases or just when invasion would occur It must be confessed that after all the years of *study and discussion there is no exact information* about the frequency with which invasive carcinoma develops But since invasive foci may develop there is little doubt that the patients usually will be treated in some way[14 60 9] A recent survey[61] of therapeutic policy in the university medical centers of this country and Canada indicates the diversity of procedures in use today Payne[9] and Younge[137] have briefly but critically discussed this problem

If treatment will be rational it is crucial to determine whether invasion has or *has not developed in the field It makes no difference how abnormal the cells* may appear or even how they behave in artificial circumstances no harm is done so long as they remain confined to surfaces not excluding glandular surfaces Metastasis from a carcinoma in situ is impossible *If* there is metastasis the stroma has been penetrated in at least one invasive focus although it may have been a small one It would *seem difficult to justify* radical operative or irradiational procedures for non invasive lesions which can be simply removed or destroyed *For every case clinically progressing to invasion there are a score* that have remained well after simple hysterectomy or even cervical amputation or cauterization When 95 per cent or more of lesions are curable by simple methods the risk of post operative ureteral fistulas after lymph node dissections and of post irradiational strictures and fistulas seems unwarranted This is then the practical importance of systematic histologic study to determine whether invasion has occurred As yet there is no substitute for this often laborious procedure

Sometimes a diagnostic cone is found to contain the entire field If no invasive focus is demonstrated it may be appropriate to do nothing more but follow the patient with exfoliative cytologic studies and repeated biopsies If the field extends to the margins of the cone a more extensive operative procedure can be done and the remainder of the cervix *studied If invasion is discovered irradiation or* radical hysterectomy or a combination of both is available after conization

If preservation of the uterus is of no moment because of age or other personal and social factors a vaginal hysterectomy an abdominal hysterectomy or the ' modified Wertheim procedure of Galvin and TeLinde may *be chosen after the diag*

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THE RECOGNITION OF CARCINOMA IN SITU OF THE CERVIX UTERI BY PAPANICOLAOU'S METHOD

By W Kenneth Cuyler, Ph D

INTRODUCTION AND DEFINITION OF EXFOLIATIVE CYTOLOGY

Exfoliative cytology is the study of cells of the epithelial layers which are desquamated as a result of necrobiosis Desquamation occurs from the exposed surface of a neoplasm as well as from the surface of normal epithelium When the cells are shed into a cavity, an opportunity is provided to collect them

The study of vaginal cytology in clinical problems is not new Epithelial elements from the vagina were studied a century ago [9] Cytologic variation in the vaginal epithelium is used today as a dependable qualitative index of estrogenic activity The characteristics of the superficial vaginal epithelium may indicate also vaginal infection and mycotic or protozoan infestation On occasions intrauterine pregnancy and threatened or incomplete abortion may be strongly suggested by cells in genital smears The vaginal pool may contain cells exfoliated from the mucosae of the entire female tract

Smears of urine were employed half a century ago[8] in an effort to diagnose cancer Approximately a quarter of a century has passed since Papanicolaou published his original communication on the presence of uterine cancer cells in vaginal smears [21] The significance of his work apparently was not fully realized by clinicians until 1943 when Papanicolaou and Traut published their monograph on the subject [2] Although this method of cancer detection is still viewed with considerable pessimism by some its value has been recognized by many critical clinical investigators who have had experience with proper application of the procedure

VALUE OF THE METHOD

In the past few years a significant number of investigators has published data sufficient to establish exfoliative cytology as an accepted field in the detection of cancer and in the study of the histogenesis of neoplasms As a result of wide recognition Papanicolaou s method of cancer detection has been applied to all body systems from which fluids can be obtained It has been employed for different purposes in a variety of different kinds of investigations

To the public health official associated with a cancer control program, the value of the method lies in its ability to uncover asymptomatic malignant neoplasms in individuals unaware of their disease Obviously cytologic studies are important to the thoroughness of cancer detection examinations provided for the public

Interpretations of exfoliated malignant tumor cells in smears strengthen clinical impressions of cancer in locations from which it is difficult or impossible to obtain tissue for biopsy prior to major operations

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included in the smear there is difficulty in properly visualizing the underlying epithelial elements

We have employed aspiration routinely to obtain material for smear study and have found it satisfactory Smears made from the endocervical canal before and after sounding are best for detecting early cervical carcinoma A small, thin metal scraper for dry surfaces, a lip of the speculum or Ayre's spatula have been used on occasion Any method which seems appropriate for the circumstances should be employed

Two smears one from the vaginal pool and one from the external cervical os are made routinely Additional preparations are made when it is thought they may be helpful This is especially applicable when there is an open lesion of the lower genital tract and when there is profuse uterine bleeding Vaginal and cervical smears are made routinely because of interest in the periodic changes in the vaginal mucosa If one were limited to making only a single preparation, the cervical smear should be used for the purpose of cancer detection In our experience, between 15 and 18 per cent of cases of squamous cell cervical carcinoma would be missed if vaginal smears alone were employed[18] In general this agrees with the experience of other investigators We have found also that in more than 60 per cent of the patients who had carcinoma in situ, the vaginal smears did not contain diagnostic cells However Achenbach *et al*[1] employ only the vaginal smear and find it quite effective in detecting cervical in situ carcinoma

It is well known that early cervical carcinoma is detected in smear studies with much less error than far advanced carcinoma In late stages of the tumor necrosis and coexistent secondary infection result in a sloughing surface which contains very few, if any intact cancer cells and from which few cells of diagnostic value are exfoliated It is probably true that the number of tumor cells desquamated decreases with increasing necrosis and accompanying inflammation Whether in addition cytolysis in the vaginal pool of those epithelial cells that are desquamated accounts for failure to detect some squamous carcinomas, by the study of vaginal pool specimens, would be difficult to demonstrate This could be the case with advanced carcinoma But in most cases of in situ carcinoma this is not significant because the epithelial surface is well preserved and the cells in the vaginal pool are not excessively exposed to bacterial and leukocytic enzymes

CLASSIFICATION OF GENITAL CYTOLOGIC SMEARS

The classification of genital smears into Types I to V has been extensively used since Papanicolaou[23] proposed it Recently increasing interest in the changes which occur in the cervical epithelium with carcinoma in situ[9,10,1 6,29,30] and with pregnancy[4,6,7,17] have led us to certain alterations in this basic classification Studies of the cervical atypicalities in pregnancy necessitated redefinition and subdivision of our Type II the category which included atypical but benign *cellular* changes[6] The subdivisions were based upon morphologic changes of individual cells of the cervical epithelium and upon the kind and degree of abnormal and/or atypical nuclear changes The atypical nuclear changes requiring segregation are found in certain pathologic states identified histologically as squamous epithelial hyperplasia endocervical squamous metaplasia or even carcinoma in situ These nuclear changes differ from the nuclear changes found in pathologic states identified histologically as cervicitis

The procedure is used commonly in obstetric and gynecologic patients for numerous reasons The lower genital tract can be explored cytologically with considerable ease and accuracy Squamous cell carcinoma of the cervix is the second most common cancer in women It can be identified as cancer in smears with less error than other genital neoplasms with the possible exception of adenocarcinoma of the cervix Usually material for biopsy can be obtained with little difficulty This diagnostic measure checks the accuracy of cytologic interpretations and has been responsible for the establishment of genital cytologic studies as part of a gynecologic examination Since it has been accepted in gynecologic and obstetric practice, cytologic interpretation has been of incalculable value in the control of cancer as a result of the detection of early, curable lesions

In addition to calling attention to early invasive cancer genital cytologic studies in women reveal abnormal cells exfoliated from non invasive carcinoma or perhaps only epithelium which has undergone metaplasia and marked cellular atypism It is generally agreed that in initiating further investigation in these cases, the cytologist contributes significantly to cancer control Therefore genital cytologic studies are being directed towards the indications of neoplastic changes which are *not advanced enough to be clinically apparent*

We have been impressed[3,5] with the accurate selection of cervices that contain histologically demonstrable abnormal epithelium not sufficiently atypical to be diagnosed pathologically as carcinoma in situ These atypical growth patterns often are found in young women The smear method is a practical way to detect such lesions with minor inconvenience to patients Through its widespread application to this problem valuable data on the genesis of neoplasia may be assembled if a sufficient number of cases be followed and repeatedly studied

COMMENTS ON TECHNIQUE

The quality of the smear preparation delivered for study greatly influences the efficiency of the cytologist and the accuracy of his interpretation of the cells in the smear[14] The quality for the most part is the combined result of two technical procedures leading to the finished product (1) making and fixing the smear and (2) staining the smear *Proper selection of material for the preparation followed by correct fixation probably is the more important* Good staining is necessary of course but once the staining process has been established there is little chance at this point of a poor preparation Papanicolaou s staining procedure[3] is recommended Slight modifications have been made in the technique[15] In our work the amounts of dyes in the triple counter stain have been reduced

The need for care in preparing a smear cannot be over emphasized It requires but a few seconds longer to make a good preparation than to make a poor one Blood necrotic debris and inflammatory exudate may so clutter a smear that the epithelial cells are quite obscured On the other hand few cells of any kind may be obtained if there is vaginal or cervical stenosis or atrophy of the mucosa In either case an interpetation is difficult or impossible With some thought and patience a satisfactory specimen usually can be obtained even in difficult circumstances

The cervical mucous plug has its value in other studies but not in cancer detection It should *be eliminated before* obtaining material from the cervical os for smear preparation It contains relatively few epithelial cells and if the mucus is

included in the smear there is difficulty in properly visualizing the underlying epithelial elements

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Our recently modified classification of genital cytologic smears into types is as follows

Type I —All epithelial elements apparently normal

Type II —Abnormal but benign cellular and nuclear changes Variations of normal cells which characterize this category include slight variations in cellular and nuclear morphology, cytoplasmic vacuolation, perinuclear vacuolation, nuclear enlargement with some degree of hyperchromatism, usually slight, the hyperchromatism is usually not of the character (lacks the density and the brightness) associated with true nuclear atypicalities increased acidophilism (Inflammatory conditions such as vulvitis, vaginitis, or cervicitis)

Type IIA —Like Type II but also slightly atypical nuclear irregularities Additional morphologic cellular changes consist primarily of evidence of increased nuclear activity, increased prominence of nuclear reticulation and granulation, and of the nucleoli, some increase in size of cell and nucleus There is a uniform staining quality of the cells associated with endocervical metaplasia This is characterized by pink perinuclear cytoplasm and a pink tint in the nucleus of an otherwise basophilic cell The cytoplasm of these cells often contains vacuoles of various sizes principally small The cells are usually basal in form but the gradations from the columnar to the squamous type may be present Hyperpigmentation of the nucleus is slight These cells may occur singly or in large numbers which compose compact sheets (Includes Type II inflammatory changes and also squamous metaplasia of the endocervix Ayre's[3] 'precancer cell complex')

Type II+ —Includes elements characterizing Types II and IIA with the addition of greater cytologic atypicalities and a greater degree of hyperchromatism These changes may be seen in endocervical metaplastic epithelium and in all layers of the portio vaginalis Marked irregularity in nuclear morphology and hyperchromatic nuclei are characteristic Irregular chromatin distribution as granules as a reticulum or as irregular clumps is common Densely hyperchromatic nuclei may be found in cells from the basal and intermediate layers They are common in superficial squamous cells These superficial cells may be extremely small or extremely large and may appear singly or in masses Their nuclei vary considerably in size not necessarily proportionate to the size of the cell but are usually regular in outline The nuclei of the extremely small cells may be elongate and narrow The staining reaction of the cells from the endocervix and from the squamous basal and intermediate layers is predominantly basophilic whereas that of the superficial squamous epithelium is predominantly acidophilic ('Superficial dyskaryosis' of Papanicolaou[24] basal cell hyperactivity of the portio epithelium of Galvin and TeLinde[10] and squamous metaplasia with marked cytologic atypicalities) In one sense a weak Type III

Type III —The more severe cytologic changes which in general cannot be distinguished from those which are associated with malignant neoplasia (Questionable carcinoma in situ carcinoma in situ or questionable invasive carcinoma)

Type IV —Elements present thought to be malignant tumor cells scarce

Type V —Elements present thought to be malignant tumor cells abundant

This revised classification has been of great aid to us in the segregation of Type III smears verified eventually by pathologic studies The classification is applicable to the genital cytology of both pregnant and non pregnant women

THE SIGNIFICANCE OF CYTOLOGIC REPORTS

It should be understood without saying that a cytologist's interpretation of carcinoma in situ should be reported only after deep consideration of the evidence. Any doubt as to the severity and extent of the atypiae should be expressed in the report. Likewise, if cytologic characters warrant, the probability of invasion should be mentioned. It should be remembered by the cytologist that his interpretation of carcinoma in situ will initiate a series of events which may be inconvenient and expensive and may result in considerable mental trauma to the patient. These should not be incurred carelessly. It is enough that a few patients will be subjected needlessly to these through errors in judgement of the cytologist even after thorough consideration of the smear's contents.

Probably the quality of the smear preparation should be described by the cytologist in the report. Without some experience, few physicians will produce smears of high quality; therefore they should expect the cytologist to acquaint them with defects in their preparations that might cause an erroneous or inconclusive diagnosis. The broader the cytologic interpretation, the better the physician will be able to judge when and what diagnostic procedures to institute. It is generally agreed that an interpretation of the cells in smear preparations should be considered as an opinion and not as a diagnosis. Therefore, no definitive treatment should be based on a cytologic report.

Cytologic reports of a benign lesion or no evidence of a lesion should be allowed to stand as such only at the physician's considered judgement. If a routine smear of satisfactory quality contains nothing suggestive of a neoplasm, it is probably significant in the absence of clinical evidence to the contrary. Negative cytologic findings in the presence of a clinically apparent lesion should not preclude further diagnostic procedures. The interpretation of a neoplastic process by a competent cytologist should be investigated by further cytologic studies and histopathologic studies until the physician is satisfied with the final result of the diagnostic efforts. Repeated findings of cytologic atypicalities suggesting a neoplastic disease in the face of negative evidence, either clinical or pathologic, necessitate continued efforts toward a diagnosis. Additional smears should be made following an equivocal report with respect to cancer.

TYPE CLASSIFICATION OF GENITAL CYTOLOGIC SMEARS IN CASES OF CARCINOMA IN SITU

The cytologic classification of smears from 112 patients with carcinoma in situ studied by us in four years and four months is shown in the accompanying table. The diagnosis in each instance was established by pathologic studies.

In 1947 no attempt was made to differentiate in situ from invasive carcinoma. Differentiation was begun in 1948. Early in 1949 we first made an unqualified interpretation of carcinoma in situ. Since that time we have classified in Type III those smears thought to represent questionable carcinoma in situ, unqualified carcinoma in situ, or carcinoma in situ with probable invasion.

Of the 112 lesions, 25 (22.3 per cent) of the smears were classified as Type V, 66 (58.9 per cent) as Type III, and 21 (18.9 per cent) were missed. These percentages can represent roughly the over-, the verified and the under-diagnoses

However, there are additional data concerning these figures, some of them are extenuating. For example, of the 21 smears in which carcinoma in situ was not interpreted (missed), no interpretation was made in 2 because of poor fixation and in 1 because of paucity of epithelial cells (Type I). Marked cytologic atypicalities (Type II+) noted in the smears of 11 patients resulted in additional cytologic studies and cervical biopsies. Of the 25 smears in which invasive carcinoma was indicated by Type IV or Type V classifications, it was thought that 9 might represent only carcinoma in situ.

TABLE IX.—CARCINOMA IN SITU (SQUAMOUS) OF CERVIX
Smear Type Distribution
(1-1-47 through 4-30-51)

Year	No Diagnosis	Types I	II	III	IV	V	Subtotals
1947			2	1		6	9
1948			4	7		2	13
1949	2	1	4	15		7	29
1950			6	37	1	7	51
1951			2	6		2	10
SUBTOTALS	2	1	18	66	1	24	
							112 TOTAL

The 66 Type III classifications can be subdivided as follows:

Sixteen were classed questionably as carcinoma; usually invasive carcinoma was considered, but not carcinoma in situ.

Seventeen were classed as carcinoma in situ with the probability of invasion.

Nineteen were classed questionably as carcinoma in situ.

Fourteen were classed unqualifiedly as carcinoma in situ.

The segregation of smears into the groups cited in this paragraph is based primarily on cell types present or absent. It shows a critical attempt to classify the abnormal cells in terms of invasive and non-invasive carcinoma. It illustrates our difficulty in identifying with certainty the cervical squamous carcinoma which will be totally intraepithelial in extent. Statistically, if we consider only 14 of our interpretations to be strictly correct, our accuracy has been 12.5 per cent, and our error 87.5 per cent. However, we think those with similar experience will agree that these figures are not a fair measure of the benefit that came to these patients as a result of further clinical and diagnostic studies initiated by the cytologic reports.

An analysis of cytologic diagnoses in cases which for some reason were not studied further by means of histologic biopsy is not given. Specimens for biopsy were not always taken in the event of a cytologic diagnosis of Type I or II, although most cytologic interpretations of Types III, IV and V could be compared with histologic diagnoses. It is clear, therefore, that the number of cases of carcinoma in situ missed by an under-diagnosis is theoretically not known. On the other hand, few over-diagnoses would be unrecognized.

THE CYTOLOGIC IDENTIFICATION OF CARCINOMA IN SITU OF THE CERVIX

As the data just presented indicate, we have been unable consistently to distinguish cervical carcinoma in situ from invasive carcinoma by means of ex-

foliative cytologic studies[5, 13] This is not surprising because similar kinds of cells are found in both and because no single kind of abnormal cell characterizes carcinoma in situ The criteria used by different investigators to identify carcinoma in situ are not the same Although their criteria vary in most instances the identifying cell can be recognized as one of those Papanicolaou associates with carcinoma in situ or carcinoma in situ with early invasion Papanicolaou s descriptions[4] of these important cytologic details follow

' The criteria used in the cytologic diagnosis of cancer are based on structural modifications of the cancer cells and of their nuclei as well as on changes in their relationship and pattern when in groups and clusters In the early stages of our work our diagnostic objective was limited to a decision as to whether malignancy was or was not present Increased knowledge and experience are now enabling us to recognize with greater confidence certain characteristic cell or smear types which in some instances make possible an evaluation of the type of the tumor as well

' One such smear type is what we refer to as superficial dyskaryosis ' Smears of this type are characterized by the presence of marked nuclear abnormalities which are, however limited chiefly to the superficial squamous cells The abnormal nuclei are enlarged and hyperchromatic Binuclated or even multinucleated cells are not uncommon A characteristic perinuclear vacuole is often to be seen Some of the abnormal cells show cornification and are distinctly acidophilic Eventually the nuclear atypia may be extended to cells of the parabasal layers

In cases exhibiting this smear type a high incidence of early carcinoma of the cervix more specifically of the intra-epithelial type has been found to exist However in a large percentage of such cases biopsies have been persistently negative and in one case at least which has been under observation for approximately ten years this condition was apparently reversible as indicated by the complete disappearance of abnormal cells from later smears We, therefore consider cases of this type as strongly suggestive of an early localized low grade malignant neoplasm of the cervix which may remain dormant over a long period of time or may even undergo regression A high percentage of parasitic infections chiefly trichomoniasis has been noted in such cases In a few instances cells of the dyskaryosis type were found intermixed with typical cancer cells in smears of cases which proved to be advanced invasive squamous cell carcinoma of the cervix This seems to indicate that eventually this condition may either become associated with or develop into a frank cervical carcinoma

The cytology of this type of lesion corresponds closely to what is known as dyskeratosis of the skin yet this term could not be applied to it very well because of the absence of typical keratinization Further study is necessary to determine the nature and significance of this distinctive cytologic picture

Another smear type frequently associated with early carcinomas of the cervix more particularly of the intraepithelial pre invasive type is the one shown in Figure 3 (compare with Fig 4)* In this there is a prevalence of abnormal cell forms of the parabasal type mostly round or oval cells with a disproportionately enlarged and activated nucleus which tends to be distinctly hyperchromatic There is considerable variability in the size of the cells which appear singly

* Papanicolaou s parabasal cell type in carcinoma in situ compared with the normal parabasal cell

scattered throughout the smear, or in small groups, but not in the large impressive clusters characteristic of advanced squamous cell carcinomas Cervical parabasal cells are usually numerous and some show pronounced vacuolization One is often tempted to venture a diagnosis of a carcinoma *in situ* on the basis of a smear of this type although the presence or absence of invasion can never be decided on the basis of a smear study alone '

The cells described by Papanicolaou as constituting 'superficial dyskaryosis' are important in the problem of detecting in situ carcinoma (Fig 52) The para basal cell which Papanicolaou modestly says often tempts one "to venture a diagnosis of carcinoma in situ" is however of greater significance in identifying preinvasive and early invasive lesions (Fig 53)

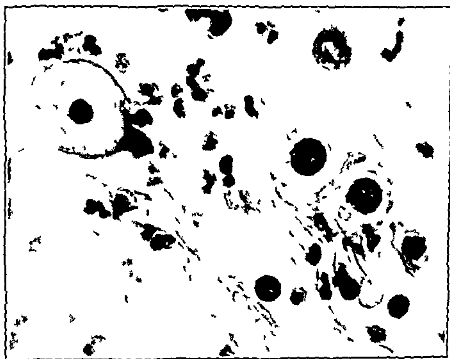


Fig 52 —*Superficial dyskaryosis associated with carcinoma in situ of the cervix* Cells from the middle layers of the superficial epithelium × 975

Ruth Graham of the Vincent Memorial Laboratory, apparently used the para basal cell to the satisfaction of her associates in the detection of carcinoma in situ The cell she refers to as third type differentiated seems to be the same as Papa nicolaou s parabasal cell A concise and accurate description of this neoplastic cell and an interpretation of its significance appears in *The Cytologic Diagnosis of Cancer* published by the staff of the Vincent Memorial Laboratory [9] and is quoted as follows

"The term 'third type differentiated is certainly not descriptive Perhaps the only attribute of the term is that it gives the number of types of differentiated malignant cells from squamous carcinoma Though the name of the cell is a poor one the cell itself is extremely important since it is the type of malignant cell which occurs in early carcinoma of the cervix These third type differentiated

cells may occur in any positive smear from a case of squamous carcinoma in addition to other cells such as undifferentiated and fiber cells However, when *only* this type of cells occurs the majority of cases show carcinoma in situ histologically rather than invasive carcinoma It should be emphasized that we do *not* attempt a diagnosis of carcinoma in situ cytologically The term carcinoma in situ necessitates an examination of the architecture of the lesion Nevertheless it appears that there is some definite correlation between early non invasive carcinoma of the cervix and the presence of third type differentiated cells exclusively This should not be interpreted as meaning that all cases of carcinoma in situ show only this type of positive cells Many of them contain every type of cancer cell On the other hand in those smears in which only third type differentiated cells are found, the tumor is usually non invasive Because these cells

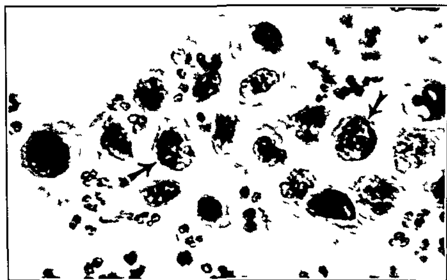


FIG 53 — *Malignant neoplastic parabasal cells in cervical carcinoma in situ* Typical forms (arrows) and morphologic variations × 975

do occur in early carcinoma, their identification is essential They are perhaps misinterpreted as benign more often than any malignant cell since they are the most differentiated and resemble normal cells more closely than any other cancer cell

Difficulties in Interpretation —The normal cell which is confused with the third type differentiated cell is the inner layer basal cell The distinction between the benign basal cell and the differentiated malignant cell depends upon nuclear structure As has been emphasized in the criteria for all types of cells from squamous carcinoma the identification of a cell as malignant relies on aberration in the nucleus In no other cell are nuclear changes so important for identification as in the third type differentiated since they are the *only* criteria upon which to base a decision The nucleus of basal cells has finely granular chromatin The nuclear border is not sharp The chromatin in the nucleus of the third type differentiated cell is irregular There are large clumps of chromatin heavy strands and fine granulations The surface of the nucleus often appears wrinkled The

nuclear border is very definite As in identification of all malignant cells if the nuclear changes are scrutinized with care the third type differentiated cells should be recognized with accuracy

' The distinction between a third type differentiated cell and an undifferentiated malignant cell rests upon the presence of a cellular border It is difficult at times to be certain whether a border exists or not It is safer to classify the cells as undifferentiated if there is any question as to whether a true border exists Undifferentiated carcinoma cells commonly occur in groups, while it is infrequent to see third type differentiated cells in groups The most exact way of determining whether cell borders exist is to see if the entire circumference of the cell is definitely outlined If one tries to distinguish definitely where one cell ends and another begins impression of presence of cellular outlines will become a certainty

'*General Criteria for Identification of Third Type Differentiated Malignant Cells* —(1) Nucleus has an irregular chromatin network There is an increase in chromatin content Nucleus often appears wrinkled Border of nucleus is very sharp (2) Cellular border is distinct The presence of a definite cellular outline *identifies the cell as a differentiated malignant one* *Cytoplasmic nuclear ratio* is often abnormal but this is not a reliable criterion, since cells may occur in which the ratio is within normal limits '

The photomicrographs and drawings which accompany this text are recommended for study Morphologic variations of the third type differentiated cell are shown

Nieburgs and Pund described[18] and illustrated[19] their diagnostic criteria for differentiating invasive carcinoma cells and non invasive carcinoma cells from normal cells The morphologic and cytologic characteristics and the staining qualities of the non invasive carcinoma cells seem to identify them with the forms often seen in the condition termed 'superficial dyskaryosis by Papanicolaou [4] More recent studies by Nieburgs [0] have enabled him to group his cytologic criteria for non invasive carcinoma into three categories Descriptions of them have been received in the form of a personal communication which is quoted as follows

Early Non invasive Cancer Cells —There are three major groups *of cells in this* category (1) These are large cells which usually have acidophilic cytoplasm *that stains yellow orange and have deep brown* pyknotic nuclei These are always enlarged They usually have well defined but irregular borders (2) These cells present a large amount of cytoplasm and have large nuclei that extend over more than 50 per cent of the cell areas The nuclei are usually somewhat transparent show few signs of activity and may have extremely fine regular granulations The borders are distinct (3) These cells are characterized by crenated nuclei which are usually small and normal in size *i e* equal to those of superficial non cornified cells They have moderate density indistinct borders and are acidophilic in reaction

These three cell types are found not infrequently in association with dissociated intraepithelial anaplasia which may eventually develop into carcinoma in situ In the larger percentage of cases however these cells indicate an early preinvasive carcinoma

" *Advanced Non invasive Cancer Cells* — These cells usually are somewhat smaller than those described above The cytoplasm is basophilic The nuclei usually but not always show a certain degree of increased activity The granulations of

the nuclei are uniform and usually are more pronounced than that which may be present in the early non invasive cancer cells Abnormal nuclear forms may be found Not infrequently double nucleated cells are present Nuclear activity may be absent in certain types of cells of this category In such cells the nuclei may be greyish in appearance and in size, comprise more than 50 per cent of the cell In rare instances still smaller cells with relatively smaller nuclei are seen These nuclei usually show condensation of their borders and a certain increased amount of transparency

Late Non invasive Cancer Cells —The morphology of these cells approximates that of cells from an early invasive cancer The cells are smaller than in the latter and are basophilic The nuclei show greater hyperchromatism than those of the advanced preinvasive cancer cells and have tendencies toward uneven granulation Abnormal cell forms may be encountered but the typical cells of differentiated squamous carcinoma are not present Multi nucleated cells and the very bizarre forms such as tadpole and spindle shaped cells and others are not present in this group

Pre invasive Cancer Cell Group —In this category in addition to cells from any, usually from all of the three groups above there is a varying number of invasive cancer cells found The latter are of the early differentiated type

"The proportion of non invasive cancer cells to invasive cancer cells may indicate the stage of transition At times only non invasive cancer cells appear in smears when biopsies reveal invasion This is due to the fact that cells from carcinoma in situ are shed more freely than those of invasive cancer The presence of invasive cancer cells in a smear is usually a definite indication of invasion

From these descriptions without illustrations we gather that Nieburgs chiefly employs variants of normal cervical superficial epithelium elements found in superficial dyskaryosis and to some extent, variations of the neoplastic parabasal cell in the detection of carcinoma in situ

Reagan [7] has given a critical and well illustrated discussion of the employment of the parabasal cell types for distinguishing carcinoma in situ He apparently does not find cells characteristic of superficial dyskaryosis helpful

Achenbach and co workers[1] have found an atypical squamous cell associated with carcinoma in situ In discussing the cytologic criteria for the diagnosis of invasive and preinvasive carcinoma of the cervix Achenbach writes as follows

We find, however that malignant cells occur with less frequency in cases of preinvasive carcinoma than in those of more advanced cervical tumors and are often accompanied by abnormal precornified squamous cells with markedly enlarged but otherwise normal nuclei These atypical anaplastic cells occurred in more than 60 per cent of these cases although they themselves are considered benign "

On Plate E of their monograph Papanicolaou and Traut illustrate 26 forms of cancer cells found in smears from a patient who had a cervical carcinoma in situ There were 7 differentiated and 19 undifferentiated forms 7 were basophilic and 19 were acidophilic in their reatcion

In the paragrahs quoted above both Graham[29] and Papanicolaou[24] stated that they do not diagnose carcinoma in situ cytologically and we cannot detect the lesion with great accuracy as already noted There are at least two reasons for this difficulty in distinguishing non invasive from invasive carcinoma by means of the cells exfoliated The first reason is that there is not a single set of abnormal

types of cells found in in situ carcinoma and another different set of abnormal types of cells found in invasive carcinoma. This conforms with histologic observations that a variety of differentiated and undifferentiated lesions are found in each category. Therefore many different varieties of abnormal cells may be exfoliated from either and some of the same varieties from both. Graham seems to agree with this for in a recent personal communication[11] she makes the following statement: "I have a very definite feeling that there are two types of vaginal smears seen in intra-epithelial carcinoma. One type has only the most differentiated cancer cells and the other type is not distinguishable from invasive carcinoma cytologically." Nevertheless, it is worthwhile to try to identify the complexes of abnormal cells usually, or often found in cases of in situ carcinoma. To some degree this can be done. Even so, there is a second reason why one could not expect to identify the earliest invasive change in a carcinoma in situ by means of the exfoliative cytologic technic. The area or areas of invasion may be quite small in comparison with the total area of the carcinoma in situ. Moreover, as Nieburgs[20] pointed out, there is less chance of exfoliation from the invasive portion than from the superficial. Therefore, in the early stages of invasion relatively few abnormal cells in the smear would come from the invasive part. This would make its detection difficult even when the invasive area differed cytologically from the non-invasive surface area. However, those who deny any cytologic dissimilarity between the invasive and non-invasive parts of a cervical carcinoma would agree with Graham[29] that the term "carcinoma in situ" connotes limitation of the epithelial proliferation to boundaries that can be defined only histologically, not cytologically.

The following statements summarize our experiences in the cytologic detection of carcinoma in situ of the cervix and are presented as a point of departure for further investigation. These observations are based on the following premise concerning the histogenesis of squamous carcinoma of the cervix: Areas or fields of squamous epithelium of the portio vaginalis or of metaplastic squamous epithelium of the endocervix are transformed into carcinoma in situ and within *such fields invasive carcinoma may arise at one or several places.* These observations are divided into three categories and concern chiefly (1) benign pathologic states associated with lesser degrees of cytologic atypism than those found in the other two categories, (2) carcinoma in situ and (3) invasive carcinoma.

Benign Pathologic States. – It is possible to recognize cellular characteristics which can be associated with squamous metaplasia of the endocervix. Abnormal changes can be seen in cells of the basal layers and the basal cell hyperactivity of Galvin and TeLinde[10] often can be identified.

Superficial dyskaryosis[4] represents true nuclear atypism in superficial squamous epithelium. Cytologically this condition is striking because of the contrast between nucleus and cytoplasm. Superficial dyskaryosis may be considered an indication to watch for additional evidence of neoplasia. It is often associated with carcinoma in situ and is not uncommon in invasive carcinoma. This agrees with Papanicolaou's findings.[4] The process of nuclear change may progress through the epithelial layer to result in carcinoma in situ and perhaps eventually in invasive carcinoma as Papanicolaou suggests.[4]

Carcinoma In Situ. – The in situ lesion can be interpreted with accuracy cytologically, perhaps *but with infrequency.* The interpretation is dependent upon the neoplastic parabasal cell of Papanicolaou.[4] An unqualified opinion of

carcinoma in situ may be presented with considerable confidence when only these cells are present or when they are found with superficial dyskaryosis (Fig 54) When superficial dyskaryosis is noted and only an occasional parabasal cell is seen, the smear is classified in Type III and the presence of carcinoma in situ is questioned The presence of various forms of malignant tumor cells in moderate numbers with the parabasal cells introduces the element of uncertainty regarding the extent of the neoplastic process The interpretation of carcinoma in situ with probable invasion or the converse may be made depending upon which cells predominate Invasive carcinoma may exist when only parabasal cells are found in smears They usually indicate an early lesion however

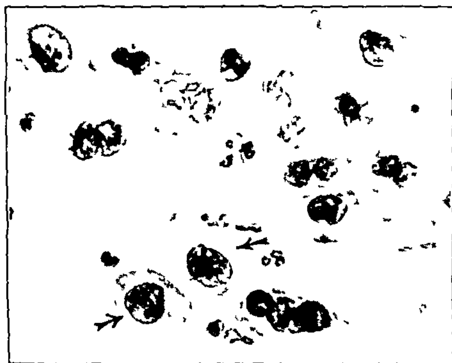


FIG 54 — *Malignant neoplastic parabasal cells* (arrows) *and superficial dyskaryosis* showing multiple nuclei and a perinuclear vacuole associated with in situ carcinoma of the cervix × 975

The parabasal cell usually appears singly but may be in clusters They are not often found in large groups This agrees with observations of other investigators [4,27] The staining reaction is predominantly basophilic Occasionally an acidophilic form is seen There may be significance in the presence of the acidophilic parabasal cell It has not been found in all smears associated with carcinoma in situ

No constant finding in any of the following characters has been associated solely with carcinoma in situ kind of nuclear granulation size form number or arrangement of nucleoli perinuclear vacuolation

Bi or multinucleation is prominent in carcinoma in situ but also is common in invasive carcinoma

18

As in the benign pathologic states but in contrast to invasive carcinoma the presence of red blood cells is not a common coincidental finding with carcinoma in situ This probably can be correlated with the absence of sufficient capillary erosion to result in noticeable bleeding

Invasive Carcinoma —It is impracticable to attempt a descriptive morphologic index of the various neoplastic forms seen in smears of squamous cell carcinoma of the cervix They can be classified according to degrees of undifferentiation or differentiation origin, or staining reaction In cataloging cell types found in carcinoma in situ none has been found, so far which does not have a counterpart in smears in cases of invasive carcinoma

It was stated above that no single cell identifies without failure the in situ lesion The same statement might be made for invasive carcinoma as well There have been instances in which an interpretation of invasive carcinoma would not have been questioned on the basis of the cells present in smears, yet serial sections of a cervical conization specimen or of an entire cervix showed the lesions to be carcinoma in situ

Free neoplastic nuclei are common in invasive carcinoma but are relatively scarce in association with the intraepithelial lesion

THE NEED FOR CORRELATIVE PATHOLOGIC STUDIES

Accurate interpretations of genital cytology in smears depend on judgement that is acquired through training and long experience studying routine smears The judgement required can hardly be gained by a course of study covering several weeks or months during which series of selected illustrative examples are provided This manner of study is a good beginning From demonstration materials alone the student could acquire some knowledge of a number of pathologic conditions after perhaps, a year s study Yet the practical problems which arise in the course of studying daily routine smears from 40 or 50 patients would not have been met The experience of studying a single problem smear for several hours comparing it with other smears and arguing over details with associates would not have been had

In a diagnostic laboratory associated with a hospital clinic each smear studied is like a practical laboratory examination or an unknown specimen The problem presented is solved and the solution is compared with the pathologic report on the concomitant biopsy specimen There is no real substitute for this critical testing of cytologic interpretations against pathologic reports One learns more from pathologic reports and review of corresponding smear preparations than from illustrations published articles lectures and demonstration slides combined Although the latter play a significant part in the curriculum of cytologic instruction they should be classed as aids in theoretical training and not as experience

The almost unlimited variations that occur in exfoliative cytologic preparations cannot be illustrated in a course of study New morphologic forms of malignant cells are seen almost daily Therefore it is constantly necessary in cytologic study to refer to the base line of histologic study A cytologist s capacity for continued development will have passed when he reaches the point where pathologic diagnoses hold no interest for him It is helpful to express interpretations as nearl as the material will permit to correspond with what one thinks the pathologist will describe from histologic studies In time, some cytologic

reports will come to be almost duplicated in the pathologic reports that follow This degree of correspondence, of course depends on the cytologist s personal acquaintance with the pathologist and his viewpoint

In the consideration of carcinoma in situ the pathologist s concepts of the lesion greatly influence the accuracy of cytologic diagnosis, if the pathologic report is accepted as the measure of correctness or incorrectness A competent cytologist knows the origin of most of the cells seen in smear preparations He will recognize degrees of abnormality in these cells However, he must have standards by which to interpret the meaning of these degrees of abnormality It would seem that these standards must be derived from those of pathologists Unfortunately, pathologists differ on what constitutes a carcinoma in situ We have no intention of entering that discussion Neither do we wish to discuss what constitutes histologic evidence of invasive carcinoma The point simply is that in these circumstances universal standards of cytologic diagnosis are not possible

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Chapter

9

NON-SILICA PNEUMONOCONIOSES

By J P Wyatt, M D

Historical Introduction —The inhalation of vapors smokes and dust with disease producing potentialities has occupied the minds of men for generations In the Renaissance, both physicians and mining engineers were aware that metal miners suffered from shortness of breath and died prematurely Anatomists described heaps of sand and stones in the lungs of granite cutters which grated on their dissecting knives Herbert Hoover, a mining engineer in 1912 translated Georgius Agricola s "De Re Metallica written in the sixteenth century and unearthed the statement that if dust has corrosive quality, it eats away the lung and implants consumption in the body '

Terminology —Following upon these dictates of antiquity, an infinite variety of definitions and terms were introduced during the nineteenth century de scribing forms of dusty lungs supposedly encountered in medical practice Names such as chalicosis or tin miner s lung rot although full of sound and fury but signifying nothing were in use at that time Later investigations revealed that the variety of stony lungs encountered in miners was due principally to the fibrosing action of uncombined silica

These attempts to sub classify dusty lungs have introduced many exotic terms for instance ptilosis is a lung condition alleged to follow the inhalation of óstrich feathers But as Merewether puts it, what this definition really means only the bird knows Despite repeated cautionary remarks on terminology and the constant need for scientific scrutiny, the premature urge to label lung conditions with fanciful phrases is still rampant

As mineral substances other than free silica give rise to industrial lung disorders the term, pneumonoconiosis coined by Zenker in 1866 meaning lung full of dust has been used in this essay in its widest sense or as Gardner used the word meaning all chronic lung changes induced by prolonged inhalation of dusts or chemicals

Industrial Hazards —The extent of industrial exposure in the dangerous dust concentrations of mining quarrying and manufacturing was estimated by Lanza and Vane in 1934 at half a million workers This in siliceous occupations alone! Acturial figures from the years 1916 to 1926 give the ratio of actual to expected tuberculosis deaths from 1 833 per cent in underground lead and zinc mines 976 per cent in granite and sandstone workers to 804 per cent in gold and silver mines indicating the overwhelming devastation of acid fast infections in troglo dytic workers In England over 800 people die each year from pneumonoconioses and 20 times that number are receiving some form of disability compensation Today because of widespread industrialization and the diversity of metals used in

industry, the potential occupational hazards are infinitely greater It is only through vigilant public health measures, engineering sanitation compensation laws and constant research that the inroads of chronic industrial pulmonary diseases are restrained Due to preventive measures, silicosis on the South African Rand in twenty years has fallen from 373 cases out of 14,887 to 26 out of 13,696

KNOWLEDGE OF SILICOSIS CONTRIBUTES TO THE WHOLE PROBLEM OF INDUSTRIAL LUNG DISEASES

Silicosis up to the 1930's —With the tragic disintegration of tin mining in Cornwall a clamant demand for an investigation of industrial conditions was answered in 1902 by an English commission The members of this commission drew attention to differences between tin miners phthisis and conventional tuberculosis In such a fashion a long series of exciting hard won investigations in the siliceous industries laid out the line of attack *to be followed in the scientific* probing into other forms of dust diseases The Rand South African workers, Watkins Pitchford Simson, Strachan and Mavrogordato, between 1914 and 1930 enunciated the koniophage transmission mechanism and the clearance of dusts via the lymphatics of the lung These workers and Haythorn in Pittsburgh noted the effects of silica upon the lung producing perilymphatic fibrosis beading and confluent scarring The experimental production of silicotic nodulation in guinea pigs was accomplished by Gardner in America and Kettle in England

Dust and Infection —These brilliant prolonged experimental works of Gardner and Kettle indicated a deleterious modus operandi in which dust was a vehicle *for pathogenic organisms especially for the omnipresent tubercle bacillus* The enhancement of tuberculosis by silica dust was investigated by these workers But the relationship between dust and infective processes has not yet been settled more recently Vorwald successor to the late Leroy Gardner has produced fulminating tuberculosis in guinea pigs exposed to quartz and simultaneously receiving BCG organisms

The Effect of Dusts on Dusts —The defeat of silicosis has been sought through antidotal chemical measures Denny and associates and later King found that finely divided aluminum dust produced a protective covering over the quartz particle depressing the solubility of silica and preventing the development of silicosis in experimental animals The results of this experiment were extrapolated to the gold mines of Northern Ontario In these hard rock regions the miners are constantly exposed to an aluminum dust atmospheres in the change houses before and after the working shifts But the prophylactic value of this maneuver is still highly debatable

Stewart and Faulds in the 1930 s showed that the iron component in the hematite lung was inert the diffuse fibrosing nodular fibrosis encountered in these hard red lungs is due to the fibrogenic action of free quartz freely liberated in iron ore mining particularly hematite and less frequently encountered in the mining of magnetite ore The inert ore trapped in the lung lymphatics produces a *diffusely* stippled radiographic plate (dust reticulation) creating shadows which are often confusing to the radiologist

Changing Concepts of Silicosis —Cummins ably studied the coal miner s lung in South Wales and later Gough of the Welsh School of Medicine brilliantly

utilizing a modification of Christeller s whole section technique applied to lungs, demonstrated a relationship between heavily carbon charged lung lymphatics (dust reticulation) and miner s asthmatic emphysema Such an exposition materially alters our beliefs about the innocuousness of anthracotic pigmentation and justifies perhaps the coal miner s term "the black twin of silicosis Belt, who left an admirable morphologic description of the silicotic tissue response (Fig 57) had previously indicated that dust reticulation of coal miners is a form of silica effect This early manifestation, dust reticulation is not seen in hard rock miners, according to Belt owing to the higher concentrations of free silica Gough attributes the fibrosis around the coal foci in long standing cases of coal miner's pneumonoconiosis to an additive infective process

An editorial in Lancet summarizes the many reasons for the interest in mining and miners becoming widespread The householder is anxious to know whether he will get enough coal to keep warm this winter the social economists are concerned with methods and results of the proposed nationalization of the mines



Fig 55



Fig 56

Fig 55 —Fibroblastic thickening of septal wall with pronounced swelling of alveolar cells present in diffuse fibrosing pneumonitis of Hamman and Rich Contrast with other photomicrographs × 108

Fig 56 —Thickened septas and profound metaplastic septal cell lining alveolar walls in cadmium smoke poisoning × 168

the industrial psychologist must estimate the effect that nationalization first in prospect and then in action will have on output But underlying all these questions is the miner himself his personal risks to limb and lung and how these can be mitigated The solution to all of these problems may well rest upon the final answer that is given on the true nature of the coal focus within the lung The study of the coal focus and related effects depends upon continuance of research into the industrial lung diseases

Contribution of Radiology —From routine radiological surveys of industry considerable information on hazardous occupations has been obtained The extent and distribution of dust concentrations and related scar reaction in radiological examinations are widely used radiographic criteria There is some correlation between the anatomical topography of dust reticulation silicotic nodulation and later confluence with the radiologists dictum of the leafless tree the tree in bud and the tree in leaf

industry, the potential occupational hazards are infinitely greater It is only through vigilant public health measures, engineering sanitation, compensation laws and constant research that the inroads of chronic industrial pulmonary diseases are restrained Due to preventive measures silicosis on the South African Rand in twenty years has fallen from 373 cases out of 14,887 to 26 out of 13,696

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are difficult to apply to particles less than 3 microns in size and yet it is these very particles that are of the greatest pathological significance As quartz is a crystal line unit of silicon and oxygen arranged in a particular way it is not enough to determine the mere existence of the element silicon by spectroscopic methods Admittedly then, all methods should be used to arrive at a critical assessment of the problem All of these methods of examination are frequently utilized nowadays to satisfy compensation demands industrial commissions and judiciary courts But of all methods the morphological analysis remains the best and represents a last court of appeal

Harrison Martland has enumerated four criteria which are basic requirements for the incrimination of a dust or poison, as the incitant of an industrial lung dis order

Postulates for Proof —1 It must be proved that the victim was exposed to a specific poison

2 The symptoms reported must be compatible with the syndrome known to result from the specific poison

3 The autopsy must disclose lesions of the type known to be caused by the poison

4 The poison must be recovered from the tissues or fluids in amounts con sidered as lethal or disease producing quantities

These measuring rods are the ideal Unfortunately because of the blunderbuss efforts of nature, it is often difficult to apply them Yet, an attempt should be made to extract and weigh the information that is available on the more recently described dusty lungs and chemical pneumonias

Although ascertainable truth under the best of circumstances is 'piecemeal fragmentary uncertain and difficult to glean an effort should be made to correlate the tissue truths as they are known today about a number of lung dis eases not directly related to silica

This background of information derived for the most part from earlier studies on silicosis offers a framework for the consideration of other injurious chemicals and dusts upon the lungs With such knowledge the pathological effects of volatile metals rare earths innocent silicates exotic plant material newly created alloys or plastics can be analyzed assessed and annealed

METALLIC PNEUMONITIDES

Acute inflammatory changes in the lung parenchyma may develop after transi tory or insulting concentrations of inorganic metals or their salts Of these beryl lium manganese cadmium and nickel are the most dangerous As beryllium gives rise to conditions which are in many respects different from those associated with other metallic irritants it will be considered in greater detail

Manganese —The ores pyrolusite braunite manganite and others contain manganese oxide and have a global distribution Because of its hardening and anti corrosive qualities manganese is alloyed with other elements to form ferro manganese and silicomanganese A manganese bronze is used extensively in marine and mining works to prevent moisture corrosion on moving parts of machinery The oxide of manganese is used for bleaching dyeing and oxidizing purposes in the manufacture of glass or textile fibers printing of cotton materials and elaboration of organic materials

Silica and the Tissues — The equation of particle size, dust concentration, duration of exposure and individual susceptibility have had unbounded reference in the literature. Equal in quantity and contention are the theories as to the mode of action of silica. The "sharp particle theory" was succeeded by the solubility theory sponsored by King, Vorwald and other workers. In this postulate the toxic action of silicon dioxide is alleged to be due to the formation of silicic acid in body tissues directly stimulating fibroblasts producing a characteristic redundant fibrosis. Heffernann modifies this solubility theory by claiming that such a rapid chemical solution requires freshly cloven silica with fragmented ends and a distinctive stereo chemical crystalline structure.

Recently Velicogna, and in this country Evans and his associates reintroduced the physical force response postulate. This thesis was put forward because certain objections to the solubility and chemical interaction theory existed. It has been known for years that (1) benign silicates provide the same or greater ion concentrations as does quartz, and yet massive fibrosis does not develop; (2) crystalline silicon dioxide (quartz) is highly active, yet amorphous silica of identical chemical composition is relatively inert; (3) fibrous tissue is no better barrier to substances in solution than the parenchymal tissue replaced, (4) that local concentrations of silicic acid have never been demonstrated. Thus a new approach to the problem of silicosis was advanced. The investigations of Evans and others took into account the peculiar properties of quartz, knowing that fibrous dusts have no *point of molecular symmetry and are capable of piezo-electricity*, whereas benign dusts are symmetrical or amorphous and mechanical or electrical influences have no effect. Although this theory is attractive, Vorwald and Parmeggiani have pointed out several defects in this peizo electric postulate.

Out of this historical pyramid of facts, and welter of diverse investigations into the action of silica, certain laboratory methods of investigation have arisen which are of scientific importance *in determining the nature of the agent or agents* responsible for an industrial lung disease.

Analysis of Lungs — In the last twenty years the available methods of assaying the foreign content of lung tissues have been greatly enlarged. These newly established procedures are invaluable for the complete investigation of any new lung disorder.

1. Microincineration (spodograms)
2. Polarized light
3. Petrographic
4. Chemical analysis
5. Spectrography
6. X ray and electron diffraction
7. Tissue studies

To this list, additional means of probing the provenance of a lung disorder may be added. Histochemical analysis, tissue culture, ultraviolet spectrography and other methods should be utilized in the complete investigation of a baffling or unique industrial lung disorder. In the final analysis of the entire problem attempts to induce the disorder in the experimental animal must be attempted.

None of these maneuvers can stand alone. Chemical analysis of the lung may give a *high silica reading*, as has been repeatedly demonstrated in industrial city dwellers, but redundant collagenous fibrosis of silica is not observed in the lung tissue. The diffuse lung alterations in chronic beryllium poisoning are a feature of the radiographic and morbid anatomical studies, but spectrographic analysis has failed in some examples to reveal the metal. Petrographic methods

The principal gas formed during this process is nickel carbonyl $Ni(CO)_4$ and was apparently inhaled by the workers

Of 8 workers in the pit, 3 died within a fortnight, from a fulminating lung disorder The onset was sudden the dyspnea intense, and the recovery of the 5 survivors was rapid The x ray examination of the chest in the 3 fatal cases showed extensive fine shadows in both lung fields In the non fatal cases, serial roentgenologic plates revealed that the changes within the lungs were reversible Vital capacity studies over a period of months confirmed this radiological observation

The morbid anatomy in these 3 cases indicates the exclusive location of the pathologic alterations within the lung parenchyma Grossly, the lungs were heavy, wet and dark red in color Histopathologic examination revealed widespread acute disorganization of the lung architecture Within the alveoli there was considerable accumulation of eosinophilic material and hyaline membranes in relation to the deranged septas Intracapillary engorgement and agglutinative red cell thrombi were plentiful Capillary rupture into the alveoli was occa

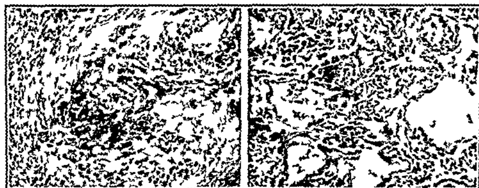


Fig 57 Fig 58

Fig 57 —Severe hyaline fibrosis of a mixed silicotic nodule with jigsaw drifts of imprisoned carbon pigment (× 108)

Fig 58 —Septal wall thickening due to mononuclear cell infiltration and connective tissue proliferation Syncytial cells enmeshed in hyaline membrane from a fatal case of nickel carbonyl poisoning (× 120)

sionally encountered The septal walls were infiltrated with lymphocytes plasma cells and a few neutrophilic stragglers The fibroblastic organization both within the swollen septal walls and budding into the alveolar spaces was a startling finding (Fig 58) This fibroblastic proliferation was quite intense by the fourteenth day

The lymph nodes other than revealing sinus reticulosis were non contributory The other viscera were free from alterative effects Scrutiny of the microscopic sections in these 3 cases indicated that the lesions observed in fatal nickel carbonyl poisoning were remarkably similar to the markings observed in an intense rheumatic pneumonitis The changes also simulated those seen in radiation pneumonitis and hypersensitive states

Other Metals Vanadium tungsten and osmium are precious metals and are increasing in usefulness in industrial fields Vanadium is of particular value in special alloy steels because of its fatigue and high thermal shock resistance proper

Although chronic manganese poisoning has been known for over a hundred years through its effects upon the substantia nigra and red nucleus of the central nervous system producing the clinical picture of shaking palsy of Parkinsonism the alterative changes in the lungs have only been recognized since 1921

The bulk of the reports on the effect of manganese upon the respiratory tract have come out of Germany The workers, principally in manganese smelting works, suffer from a form of lobar pneumonia, known in worker s parlance as "manganese flux pneumonia" Compensation for this basic slag pneumonia is given in Germany Baader in 1933 blamed the high incidence of pneumonia among workers on dry cell batteries upon the manganese component In Sauda, Norway the continuing high incidence of pneumococcal pneumonia is ascribed to the overhanging manganese smog

No characteristic patterns of morbid anatomy have been described in the few fatal cases subjected to post mortem studies Lloyd Davies recorded the fact that pneumonia in workmen employed in atmospheres heavily laden with particles less than 1 to 15 microns was no different than the current pneumonias in the local district but that the incidence was far, far greater No permanent effects could be demonstrated by radiographic studies

Exposure of mice to manganese dust produced a diffuse, bilateral necrotizing pneumonitis in which the dominant infiltrative cell was the mononuclear Capillary rupture and spilling of red cells into the alveoli accompanied the septal cellular infiltrations Consolidation and intra alveolar organization was a terminal alteration On the basis of the pathological changes observed in the mouse lung it would be difficult to distinguish a manganese pneumonitis from current atypical pneumonias

Cadmium —Paterson, in 1947 and earlier Frankish observed in several instances striking lung alterations in workers dying from acute respiratory disease These men engaged in welding operations, had inhaled intense concentrations of cadmium smoke Death occurred within a few days and the lesions observed were of a spectacular character and previously unrecognized

In the early fatal cases profound capillary engorgement intra alveolar protein edema fluid infiltrates of mononuclear cells were noted within the septa and in the lungs of men who had lived more than five days there was a remarkable proliferation of the bronchiolar lining cells In this diffuse indurative pneumonitis the septal cells in an active proliferative phase have undergone a transmutation into a stratified squamous metaplastic stage and grown down in a vigorous fashion into the alveoli (Fig 56) Collagenic fibrosis was focal and of a stellate pattern within the air sacs The majority of the men recovered from this episode and subsequent examination did not reveal permanent pulmonary scarring Several pathologists have submitted for consultation additional examples of fatal cadmium smoke pneumonitis with the lungs presenting the highly characteristic alterations originally depicted by Paterson

Paterson s studies on the toxicity of inhaled cadmium revealed that an analogous condition can be readily produced in rats

Nickel —Sutherland Whittaker and Penny noted the pathologic effects of a combined gas smoke upon the lung parenchyma In an industrial accident in 1948 during the clarification of nickel in the Mond process, the gas evolved escaped into the workers pits through failure of the furnace ventilative methods

metal or the individual acid radicals as the toxic agent in the production of this unusual pulmonary disease Or as a Lancet editorial says, "to charge such an admirable metal with having poisonous properties is about as distasteful as accusing a trusted butler of stealing the family plate

Althouth non toxic halophosphate substitutes for beryllium in the fluorescent lamp phosphors have been introduced, it is still necessary as an alloy in metals and therefore this form of poisoning will remain a problem

Etiology —The inhalation of beryllium produces a major disturbance in certain individuals either directly or through a chain of events which develop in the lung following previous sensitization As the primary concern in this contribution is the sequence of pathological changes within the lung only passing reference to skin liver, lymph node and osseous involvement is made The cutaneous lesions are apparently of the direct contact variety the liver and skeletal involvement in animals are systemic manifestations the changes in the root lymph nodes are directly related to lung drainage

Pathogenesis —Beryllium poisoning exists in two forms acute and chronic associated with dermal and respiratory manifestations These two forms of disease have no mutual relationship although Machle has insisted that no differentiation between acute and chronic forms can be made The differences between the acuity and chronicity of the processes being attributable to the solubility of the compounds to which the individuals are exposed Probably, the more soluble compounds of beryllium containing a strong electronegative group, such as fluorides or halides are implicated in the acute cases requiring only a few moments of exposure The less soluble compounds apparently cause the chronic disease

Clinical Findings —A contact dermatitis in susceptible individuals developing in seven to fourteen days after initial exposure or indolent ulcers following abrasions of the knuckles may be cutaneous warnings of hypersensitivity Other individuals develop an acute nasopharyngitis or tracheo bronchitis which may blow up into the dreaded acute chemical pneumonitis or beryllium poisoning Acute beryllosis is most common in the processing and machining stages with 310 acute cases and only 15 chronic cases reported by Machle

The acute pneumonitis is of sudden onset with substernal pain and progressive shortness of breath The diffuse bilateral haziness of the roentgenographic plate is not seen till two or three weeks after onset of symptoms Of 98 cases Van Ordstrand investigated 12 were fatal Dutra states that the mortality rate in his series was 1 per cent the mortality is usually reported as 10 to 20 per cent Deaths occurred between twenty and thirty days after onset of illness Survivors of the acute pneumonitis followed up for a period of ten years have completely recovered and no residual changes can be demonstrated by radiological or clinical studies

Biochemical and Hematological Values —In chronic beryllium poisoning the plasma proteins are elevated particularly the alpha and gamma portions of the globulin fraction and the profile of liver function tests shows variable deviations No characteristic change in the peripheral blood or marrow is noted

Pathological Anatomy of Acute Pneumonitis —The lungs in fatal cases of acute beryllium pneumonitis are unusually heavy weighing over 1000 grams each Cut surfaces are homogeneous edematous and overcast with a grayish tinge Pus cannot be expressed from the lung The bronchial lymph nodes are soft and succulent

ties Although a number of instances of vanadium poisoning with the possible induction of a pneumonitis have been reported further investigation of vanadium and the lesser used metal osmium is required No well documented pathological reports on the state of the lung are available Tungsten warrants a closer scrutiny for possible permanent lung effects

The effects of arsenic, salts of chromic acid and ionizing radiation are not discussed in this contribution, being of a nature that places them beyond the stipulated scope Although the principal action of these agents is through the insidious induction of neoplasia, no well-established bridging pneumonoconiosis has been established for these agents

Beryllium —*Historical Introduction* —This rare metal was first extracted from ores in 1931 Shortly thereafter a bronchiolitis was observed in workers in Germany (engaged in such a labor) by Weber and Engelhardt In rapid succession between 1935 and 1943 Fabroni, Gelman Berkovits and Izrael Meyer and Van Ordstrand described various forms of this 'metal fume fever' A subsequent review by Martland of his medico legal material indicates that beryllium poisoning existed in New Jersey in 1934 With the incorporation of beryllium into phosphors of fluorescent lamps in 1938 the lung disease became acute and disturbing and being first observed in Massachusetts in the early fall of 1941

Properties and Uses of Beryllium —Beryllium, because of its unusual physical and chemical properties, has been called the "glamour child of metallurgy' or the 'Admirable Crichton of metals' As beryllium is about as light as magnesium possesses a modulus of elasticity greater than that of steel and a melting point far higher than that of aluminum, and because of these extreme properties, it finds a wide industrial usage

The oxide of beryllium is most frequently used in the manufacture of crucible and electric furnace refractories The addition of 2 per cent of beryllium oxide to a zinc silicate lacquer broadens the zinc silicate band of fluorescence under an ultraviolet lamp creating a valuable phosphor Fusion with copper produces an alloy of great strength highly resistant to corrosion and fatigue with high thermal conductivity non sparkling and non magnetic Because of these qualities the metal is heavily demanded in the manufacturing of precision tools electric welding and wiring With beryllium being many times more penetrable to x rays than many metals it is suitable for x ray windows Under nuclear bombardment beryllium and its oxide are excellent metallic sources for neutrons Owing to its great elasticity and low density beryllium is valuable in radio and television tubes and acoustic apparatus It possesses a scavenger action which is utilized in the aircraft industry to clean up aluminum alloys Because of mechanical stability it is used in aircraft pistons

Because of these features beryllium is widely used in the ceramic industry neon incandescent and fluorescent lamp manufacturing casting of beryllium copper alloys precision tooling electric welding and wiring manufacturing of heating elements radio and television tube production aircraft industry and atomic energy works

The widely used oxide of beryllium is extracted under high temperatures from crushed beryl, an inert ore by concentrated acids and fusions In addition to the use of the pure metal and the oxide beryllium has been bonded with other acid salts such as fluorides nitrates nitrides silicates and stearates From this large number of beryllium compounds it is difficult to selectively incriminate the

fever, and later cyanosis and digital clubbing herald the 'delayed chemical pneumonitis of beryllium workers' Most of the chronic cases occur in fluorescent lamp workers workers engaged in alloying beryllium and the singular group of so called neighborhood cases' This latter group refers to the development of the chronic disorder in individuals not occupied in the industry, but living in the neighborhood or in the same household as a beryllium worker The exposure time in these individuals is completely variable and apparently individual differences play an important part Certainly the neighborhood cases suggest an allergic" background

Pathological Alterations in Beryllium Granulomatosis —The lungs in chronic beryllium granulomatosis are heavy inelastic and the combined weights range around 1500 gms Cut sections reveal many firm nodules measuring up to 4 mm in diameter fused into a diffuse honeycombed background Emphysematous regions subpleural in situation and within the parenchyma are associated with dense trabecular strands of scar tissue The regional lymph nodes are firm and prominent The two other interesting observations are the inevitable accompaniment of thickening of the right ventricle and according to Van Ordstrand 20 per cent of the cases posses renal stones containing calcium, and in some instances beryllium

To Dutra the chronic granulomatosis is a morphological extension of the acute pneumonitis There is a carry over of the mononuclear exudate, particularly within the septal walls The startling feature in the chronic form of the disease is the existence of fresh non_caseating regressing or fibrosing granulomas within the septas perivascular and peribronchial tissues (Fig 60) The centers of the granulomas are composed of granular, eosinophilic debris accompanied by mononuclears and Langhan's giant cells Fibroblasts are arranged around the periphery in a radiate fashion Within the granulomas and usually outside of the giant cells are numerous, irregular spherical or conchoidal bodies (Fig 61) These structures are identical with those described in the lesions of sarcoid being blue black in color in hematoxylin and eosin preparations and heavily encrusted with iron (Fig 62) Some of these granulomas become completely fibrosed, and a few show hyalinization With the coalescence of these nodules concurrent fibroblastic proliferation with intense scarring and hyalinization presents an unusual picture of active granulomatous lesions focal hyaline nodules and extreme distortion of lung tissue due to contraction of scar tissue and marked emphysema The pulmonary arteries show obliterating endarteritis and foam cell atherosclerosis

The root lymph nodes are the morphological mirror of the lung

Several instances of extensive fibrosis and granulomatosis have been noted in the liver Death occurs in one third of the cases of chronic granulomatosis and in most instances is due to pulmonary heart disease based upon alteration of the pulmonary vascular bed secondary to the extensive fibrosis and emphysema

Because of a certain amount of confusion which exists in the literature between chronic berylliosis and sarcoidosis Table X is added listing the characteristic features of each of these granulomatous lesions

The distinction between miliary caseating tubercles the acellular vitreous balls of silica the necrotic granulomas of lymphopathia venereum and a histoplasmic granulomatous reaction is not difficult

Animal Studies —There is some evidence that the acute disease is reproducible in animals Aldridge found that the lethal dose of beryllium injected intraven

In the acute form of the disease, a homogeneous, eosinophilic protein fluid fills many of the alveoli Hyaline membranes are plastered against the septal walls in many instances Fibrinoid material, apparently related to the hyaline membranes and septal walls, is present in some examples Organization of this material by macrophages is frequently observed (Fig 59)

Within the alveoli, numerous mononuclears, plasma cells, and scattered lymphocytes are contained in the granular coagulum Lipid debris or nuclear remanants within many of these macrophagic mononuclears may be noted A heavy infiltration of the septal walls by histiocytic elements is a dominant feature Another lesion is the cuboidal swelling of the alveolar lining cells These cells frequently coalesce forming syncytial cells and combined with fibroblasts creating buds of proliferating immature granulomas

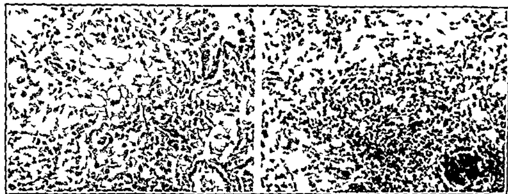


Fig 59 Fig 60

Fig 59 —Profound disorganization of lung in acute pneumonitis of Beryllium poisoning Protein edema fluid swollen septas with mononuclear infiltrates and cuboidal septal cell (× 156)

Fig 60 —Diffuse mononuclear response with collagen scar tissue Regressing granuloma and giant cells at periphery of mononuclear response both features of chronic granulomatosis in Beryllium workers (× 108)

Capillary engorgement and erythrocytic extravasation into the alveoli is present A metaplastic change of the terminal bronchiolar lining has also been described which may follow the embryonalization of the septal cells

The related lymph nodes are stuffed with mononuclear phagocytes resembling those seen in the alveolar exudate The lymphoid germinal centers of Flemming show ' toxic alterations Dutra Vorwald and Martland have described in isolated instances centrilobular necrosis of the liver and unit granulomas composed of lymphocytes and plasma cells surrounding cores of amorphous eosinophilic debris

Death in this phase of the disease is due to asphyxia and acute pulmonary insufficiency related to the intense edema and intra alveolar fibrinoid change interfering with gaseous exchange Cor pulmonale is unusual in the acute phase

Chronic Granulomatosis of Beryllium Workers —*Clinical Findings* —In contrast to the acute form the chronic variety is insidious and delayed in onset sometimes as long as ten years after initial exposure The exposure may be slight or prolonged The radiological appearance presents a diffuse symmetrical and fine miliary stippling and is frequently noted months before symptoms occur The symptoms of fatigue weight loss cough increasing dyspnea on exertion and

smoke and nickel carbonyl poisoning Reviewing my material and that loaned by Paterson Whittaker and Penny, it was noted that both of these metallic poisons produced alterations remarkably similar to the acute lesions of beryllium pneumonitis These morphologic findings may not be so surprising when it is considered that beryllium inhibits alkaline phosphatase in the septas by competition for magnesium The absence of phosphatase has been noted through histochemical observations on experimental animal tissues Denz has postulated a similar effect of metals upon respiratory enzymes following his experimental investigations with nickel carbonyl Furthermore Altschuler has indicated that in a variety of acute conditions the fibrinoid change is apparently a reflection of derangement in acid polysaccharides within the septal structures Whether this same speculative mechanism accounts for similar pathologic alterations seen in fulminating rheumatic lung disease or in the lungs of individuals dead from the combined effects of disseminated lupus erythematosus and sensitizing blood transfusions is conjectural But under the microscope distinction between all of these conditions is often difficult, because of their morphological similarity

Further experimental support for the interference of alkaline phosphatase enzyme system is suggested through the production in rabbits of beryllium 'rickets and the development of autonomous osteogenic sarcoma following intravenous injection Apparently radioactive beryllium enters bone after exceeding the tolerance of excretion visits the osteoblasts remains at that site profoundly disturbing this highly important enzyme system of bone The inhibition of alkaline phosphatase in the liver may account for the clinical picture of systemic toxicity Such a selective enzyme derangement by beryllium may be the trigger mechanism for the tissue response finale

Van Ordstrand has noted positive skin tests in the acute forms of the disease suggesting a hypersensitive status

In the chronic form of beryllium granulomatosis because of its lengthy developmental maturation pattern and because of this metal's property of producing metastasizing osteogenic sarcoma in rabbits the possibility of the initiation and development of bronchial carcinoma although remote must be considered in humans To date no acceptable evidence of such a mutual relationship is available No increased incidence of tuberculosis has been observed in chronic lung disease of beryllium workers

The ACTH hormone of the pituitary has been used with some success in known cases of chronic beryllium granulomatosis Because of the known anti inflammatory properties of ACTH with its power to polymerize cement substance future morbid anatomical studies in ACTH treated cases will be of value

The conchoidal bodies identical to Schaumann's inclusions of sarcoidosis, were originally regarded as highly characteristic Their presence in the granulomas of the fatal cases from the beryllium manufacturing plants in Salem Massachusetts partially accounted for the chronic granulomatosis of beryllium being given the witches name of Salem sarcoid Martland's medicolegal case of 1934 prominently displays these singular structures In that these spiculated and calcific inclusions have been noted in genuine cases of Boeck's sarcoid and other disorders of the reticulo-endothelial system with the common denominator of hyperglobulinemia the nativity and structure of these bodies may be related to this biochemical lesion of increased blood globulin The intense mononuclear septal pneumonitis in beryllium poisoning

ously in rabbits, rats and mice, as solutions of ionized salts, is between 0 5 and 1 0 mgm per kilogram Death occurred in one to three days and autopsies showed liver, kidney and spleen lesions Between 25 to 30 per cent of the beryllium localized in the liver, and was fixed to tissue proteins Localization and fixation to body protein in the skin and lungs occurred after subcutaneous and inhalation administrations Scott, using beryllium sulphate, through inhalation and intravenous routes produced a bronchiolar edema and macrophagic response None of the morphologic findings of a mononuclear septal pneumonia were produced Hodge with intraperitoneal injections of beryllium compounds evoked a mononuclear response but was unable to convert the response to a granulomatous reaction

TABLE X —DIFFERENTIATION BETWEEN THE LESION IN SARCOIDOSIS AND THE BERYLLIUM GRANULOMA

	Beryllium Granuloma	*Sarcoid*
1	Sparse epithelioid cells	Nests of compact epithelioid cells in the pink of condition
2	All phases of progression	Granulomas all of same age
3	Small giant cells at periphery of lesion	Large central giant cell
4	Septal fibrosis present	Regressing nodule of sarcoid surrounded by court of hyaline fibrosis
5	Numerous infiltrative mononuclears present	Peripheral placement of lymphocytes
6	Granular cores of granuloma	No necrotic centers
7	Beryllium may be determined in the lung	Negative

Beryllium compounds in many forms and along many routes have been investigated by Vorwald, and he states that these compounds have been studied under a ' great variety of conditions, such as diet dust inhalation during pregnancy and the effect of infection in an attempt to reproduce the type of tissue reaction present in human cases In no instance however have we succeeded

Toxicological Investigations —Both chemical and spectrographic evidence has accumulated in the pneumonitis and granulomatosis of beryllium workers that beryllium can be quantitatively determined The original chemical determinations on tissue from beryllium workers were hindered through lack of a delicate method of determination Unlike the chemical components in silicosis asbestosis and other pneumonoconioses the beryllium content determinations have been minute quantities expressed as micrograms per 100 gm of tissue In some instances even after the entire lung specimen has been ashed a positive reading of beryllium cannot be obtained During life Dutra has emphasized that the beryllium eliminated in the urine should be assayed

The point has been emphasized by Elkins that if we are to consider that an atom of beryllium is as harmful as an atom of lead the relative atomic weights must be regarded Beryllium has an atomic weight somewhat less than one twentieth the mass of lead which possesses an atomic weight of 207 This means that a section of bone or other tissue containing 1 microgram of lead may be considered as no more abnormal than a similar section holding somewhat less than 0 05 micrograms of beryllium Therefore a chemical method sensitive to hundredths of a microgram is needed These conditions are now being met by Cholak and Hubbard s method

Discussion —The lesions of acute pneumonitis of beryllium workers are not specific Analogous lesions have been seen in fatal human cases of cadmium

Silver —In silver polishings with constant use of rouge iron oxide Harding and Barrie have demonstrated dust reticulation in the lungs of four silver finishers—the condition was named argyro-siderosis These workers had died from bronchopneumonia or after surgical operations unrelated to their occupation The iron oxide was deposited in the lung lymphatics the silver component in the medial wall of the arteries and alveoli No fibrotic changes of a collagenic or reticular nature were noted Orcutt in his studies was unable to demonstrate collagen formation in experimental argyrosis

Tin – The accumulation of the tin oxide within the lymphatics of the lung is another example of the accumulation of dust phagocytes leading to a benign pneumonoconiosis This state is refered to as stannosis and in every aspect is similar to the other conditions concerned with the accumulation of inert dusts in the lungs (Fig 63)

Fig 61



Fig 62



Fig 63

Fig 61 —Bizarre calcified and iron encrusted inclusional structure within a nest of mononuclear histiocytes (× 288)

Fig 62 —Irregular knobbed inclusion formation surrounded by lymphocytes and plasma cells (× 108)

Fig 63 —Accumulation of inert dust in intra alveolar macrophages in experimental animal No fibrosis present (× 108)

There is some justification for the term benign pneumonoconiosis introduced by Pendergrass and Leopold in that inert dusts may cause characteristic radiographic changes but no fibrosis But as the lung is essentially an elastic organ and if its elasticity is impaired through intracellular deposition of metals such as silver or stretched through accumulation of inert dusts in the lung lymphatics there may be emphysema and shortness of breath It is on this very point that Gough and Heppleston claim that the coal miner s asthma is due to the distortion and splinting of elastic tissue through the excessive accumulation of benign dusts such as carbon leading to permanent emphysema The opposing concept held for years has been of course that the koniophage takes up a mixed load of silica and an inert dust such as coal or iron and frequently the Odyssey of the macrophage is halted half way with the macrophage migrating through the lymphatic wall into the surrounding alveolar walls ensuring the fibrogenic action of the transported silica

and biochemical finding of high blood globulins recalls the fact that abnormally high globulins are frequently associated with plasma cell infiltrations as in myelomatosis Campbell, Good, Kolouch and other investigators have related intracellular globulin formation to mononuclears based upon a variety of experimental methods

A large number of questions remain unanswered in the beryllium problem With large numbers of workers exposed to the metal why is only a small group affected? Why a florid disease in one individual and delayed for years in another? Why the failure to produce the chronic disease in small animals if beryllium is the causative agent? Is it beryllium or a radical which produces the disease? Despite the lack of definitive answers for many questions it is satisfying to agree with Aub that we have a better understanding of beryllium in five years than we had of lead in a thousand '

THE ACCUMULATION OF INERT DUSTS

Metals and salts of metals owing to varying degrees of radio opacity, produce x ray shadows The intensity of the radiographic shadow varies with the atomic weight of the metal Arrigoni and later Pendergrass showed that the inhalation of barium dust produces extremely dense punctuate radiographic changes

These metals trapped in the lymphatics of the lung produce stippling of the lung fields and are demonstrable by radiographic studies The condition produced has been described as benign or inert pneumoconiosis

Iron —Heavy iron dust concentrations are produced in oxyacetylene grinding of steel alloys and ocher mill turneries the workers in these industries inhaling dust made up of 98 per cent iron The inhalation of iron with an atomic weight of 57 produces finer and less dense radiographic stippling than that of barium which possesses an atomic weight of 137

Since the exposition by Doig and McLaughlin it has been repeatedly demonstrated through radiographic means that iron produces reticulation The condition has been labelled siderosis This radiological term reticulation was re evaluated in the light of autopsy findings reported by Enzer and Sander Harding s experimental studies with red iron oxide injected into rats and the occasional early example of siderosis in hematite and magnetite miners unequivocally established the iron dust as lying trapped in the lymphoid nodules of the septas perivascular and peribronchial lymphatics In none of these examples has the virginal imprisoned iron evoked a fibrosing reaction

The inert nature of iron is corroborated by tissue evidence from other conditions Transfusional siderosis is in most instances unaccompanied by fibrotic reaction In the few instances in the liver in which fibrosis was found this diffuse scar reaction was conditioned by severe anemia and nutritional deficiency One patient has received 125 0 gm of whole blood iron without ensuant fibrosis In the natural occurring condition idiopathic pulmonary hemo siderosis (Ceelen Gellerstedt syndrome) the septal thickening probably represents a mesenchymal defect

Long exposures with absence of functional impairment and reversibility of radiographic shadows on removal of the worker from iron dust concentrations supports the postulate that iron or iron salts are without fibrosive powers and are constantly being cleared from the lungs

lung constituted a serious industrial menace The workers in Germany were engaged in the manufacturing of incendiary mixtures explosives and paints In Germany the condition was first described by Goralewski in 1939 Subsequent evidence indicates that the clinical pathological syndromes which developed in Germany and in Canada, although associated with different end manufacturing processes are apparently identical

Properties of the Metal —In the mining and crushing of bauxite ore in British Guiana or Arkansas no ill effects have been noted No dust is liberated at this phase of the processing due to the moisture of the ore earth The crushed ore is subsequently dried and loaded Again at this step no deleterious effects have been recorded In Canada the workers who develop the disease are engaged in the processing of bauxite for the manufacture of artificial corundum The processing is carried out in electric furnaces with the mix consisting of finely ground bauxite iron and coke Carbon electrodes are lowered to the surface of the mix and fusion occurs at a temperature of 2000 C During this process dense white fumes composed of amorphous silica and alumina are evolved and carried upwards to the roof of the factory Above the furnace pots in this heavily contaminated atmosphere are the furnace feeders and crane operators All the fatal cases occurred in this class of workers

In Germany in the manufacture of explosives an aluminum powder is manufactured either by a blowing or stamping process In such processes the aluminum particles are covered with paraffin like substances and the atmosphere is heavily contaminated with paraffin covered finely particulate aluminum oxide powder The dust concentration reached such high levels that explosions within the factories frequently occurred

Clinical Findings —Both in Germany and Canada there was a rapid development of respiratory illness, often within three or six months of commencement of work The workers complained of acute shortness of breath substernal pain and fatigue Cyanosis and spontaneous pneumothoraces resulting from rupture of gigantic emphysematous bullae were frequently noted Radiographic shadows were bilateral diffuse lace like and granular with greatly increased width of mediastinum and lung collapse At the time of Shaver s initial roentgenographic survey of 344 workers employed 17 per cent of those were effected The mortality rate with two years clinical history is approximately 30 per cent

Etiology —As chemical investigation of the flue conditions and final analyses of the lungs revealed proportional constituents with silica reading from 30 to 45 per cent and aluminum from 40 to 60 per cent of the total, both elements have to be considered in the causation of this unique lung disorder Spectographic determinations and *x* ray diffraction revealed that the majority of the particles were amorphous In the disease in Germany Goralewski Jotten Jager and Eickhoff have suggested that the mechanism is that while aluminum is highly resistant to aerial oxidation it is freely soluble in sodium chloride solution forming a sodium aluminumate in lung tissue Thus a colloidal aluminum hydroxide complex results if the sodium and chloride ions are allowed to diffuse away If proteins are also present they are precipitated around the partly dissolved aluminum particles

Pathogenesis —The German investigators believe that an ionic complex or paraffin coated aluminum initiated lung changes On the other hand it is our belief and Koelsch s that more direct factors are responsible because of the intensity of exposure and of unsatisfactory ventilation in the furnace rooms The

Perry's conclusions and my own on inert dusts are in sympathy with Gough's on the role of carbon in the production of pulmonary disability "It seems possible that any dust, no matter how innocuous in small concentrations would in large enough quantity eventually overwhelm the defences of the lung and accumulate in such amounts as to impair function, such a form of lung disease would be the result of causes of a mechanical nature—the physical presence of large amounts of inert foreign material"

Graphite —Graphite is a stereo chemical modification of the crystalline structure of carbon it is a half brother to the diamond and carbon black Graphite is used in foundries electrodes and pigments imported from Ceylon and Madagascar, and in unloading a dusty black atmosphere is created Dunner and Bagnall claim the development of a graphite pneumonoconiosis in dock workers engaged in unloading ships laden with graphite But, the siliceous content of the graphite reached as high as 30 per cent and although black delimited necrotic cavities were found in the autopsied cases, the interpretation that graphite produces a distinctive pneumonoconiosis should be viewed with caution as in the past both Gardner and Belt have stressed the frequency of aseptic cavitary necrosis in complicated silicosis On the other hand Jaffe and MacMahon have recently documented cases of graphite pneumonoconiosis which support Gough's claims on the effect of overwhelming accumulations of carbon or related pigments

Discussion —There are several points to be remembered in the interpretation of so called inert dusts (1) An intimate detailed industrial history should be obtained from the patient, relatives work mates and employers accounting for every period in the individual's occupational history (2) It should be remembered that a combination of an inert dust with fibrosive silica often occurs The relationship of inert dust to a fibrosing dust occurs not only in some instances of graphite pneumonoconiosis but also in "Boiler Scaler's disease"—a combination of iron oxide and silica Both components produce radiographic changes but the silica is responsible for the frank collagenic nodules (3) From diamond dust to marble the two defense mechanisms of the lung the cilia and the dust phagocytes (koniophages) are of prime importance in the daily elimination of particles Phagocytosis of dust particles by macrophages with intra alveolar concentration migration of dust charged macrophages into perivascular lymphatic vessels and a minimal accumulation of lymphocytes and the occasional giant cell laden with dust are the morphological reflections of non toxic dusts Beyond this any associated connective tissue proliferation is regarded as a proliferative response to toxic dusts It is in this dim borderland between massive accumulation *of dust phagocytes and early connective tissue response* that the contention arises between labelling a dust as an irritant or non irritant

BAUXITE FUME PNEUMONOCONIOSIS

Historical —In an industrial survey of workers in an aluminum abrasive manufacturing plant of Niagara Falls Canada Shaver noted roentgenologic shadows in the lung fields that were so startling and different that the films were immediately characterized as being of a distinctive origin This description constitutes the first recognition on this continent of this specific crippling lung disorder associated with the manufacture of alumina abrasives With the completion of World War II information and evidence came out of Germany that an "aluminum dust

64) In the older cases radiating bands and masses of rubbery black tissue are evident Grossly there is complete absence of shotty or confluent nodulation The gun metal color is apparently due to the diffuse scarring spreading out the anthracotic pigment The other outstanding feature associated with this pulmonary fibrosis is the presence of diffuse emphysematous vesicles The vesicles *are found not only within the fibrous parenchyma but particularly* beneath the subpleural regions Microscopically there is a constant anatomic pattern of



Fig 65



Fig 66

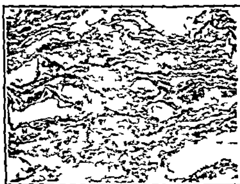


Fig 67

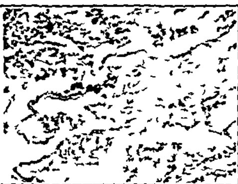


Fig 68

Fig 65 —Early fibroblastic proliferation within septal walls and beginning fusion Lymphocytes and fine particulate dust contained within swollen alveolar walls in Bauxite fume disorder (× 126)

Fig 66 —Intrinsic hyalinization of septal walls in another example of Shaver s disease (Bauxite fume pneumonoconiosis) (× 108)

Fig 67 —Melting fusion and conversion of alveolar walls into irreversible stage of Bauxite lung (× 108)

Fig 68 —Vitreous hyalinization of alveolar wall and accumulation of dust laden macrophages (× 108)

diffuse fibrosis The initial lesion is an intracellular septal edema with early fibroblastic proliferation with infiltration of lymphocytes and plasma cells (Fig 65) Succeeding the inflammatory cell infiltration there is intense fibroblastic proliferation and heavy collagen deposition The irregular starched and stiffened trabeculae are made up of characteristically sclerosed alveolar walls fre*quently suggestive of Chinese lettering (Fig 66)* The fibrosing septal walls are

mechanism is that of an amorphous dust evoking a rapidly sclerosing process within *the septum and interfering with the koniophage transmission* mechanism The absence of amorphous silica in the German factories manufacturing aluminum dust substantiates the thesis that aluminum dust in intense concentration and in an *amorphous form acts as the dominant agent, rather than the concentration* of aluminum and silica acting together The combination of dust in these cases



FIG 64 —Transection of lung in fatal Bauxite fume pneumonoconiosis Fine reticular back ground with beginning emphysema No enlargement of lymph nodes or pleural thickening

does not play a vital role in the etiology of this dust disease Although it is known that a combination of dusts will to a certain extent modify a frank silicotic process they will never completely erase it Similarly King has demonstrated that an antidotal mixture of aluminum hydroxide with quartz lowers solubility but it does not prevent experimental silicosis

Pathological Anatomy The outstanding features of the lung from the gross examination were *the relatively normal size and the gun metal color* The lungs weigh approximately 500 to 700 gm in all the cases examined There is a diffuse widespread induration throughout which produces a fine fish net pattern (Fig

tective action, as in this form the alumina is unable to take up water and offer a protective surface covering over the irritating silica particles

Denny and others in 1939 exposed rabbits to metallic aluminum dust below 5 to 15 microns in size and no fibrosis was produced Belt and King repeated these experiments in rats, and balls of fibrous tissue were formed Gardner contended that amorphous silica was rapidly eliminated and no lesions in the lung developed but conflicting evidence on this point has recently appeared from the same laboratory Utilizing the stack fumes which gave a spectrographic pattern similar to the lung residue, there are some indications that a fine diffuse fibrosis can be produced in the rat and guinea pig

Chemical Analyses of the Lungs —Spectrographic determinations revealed that the majority of the particles are not greater than 0 5 microns in diameter Analysis by *x* ray diffraction and electron microscopy shows the fume particles are between 0 01 and 0 5 microns in size in other words of submicroscopic structure

In the fume and ashed residue of the lungs beta and gamma alumina are present indicative that the alumina is present in an uncombined form Cristobalite was also found in the free silica component suggestive of the fact that the quartz had been heated to high temperatures Quantitative chemical assessment revealed in 9 lungs of our series that the distribution of silica and alumina in the ash was equal approximately 40 per cent of each These figures were of the same magnitude as the quantities within the stack fumes

RESPIRATORY DISEASE DUE TO INHALED VEGETABLE MATERIAL

In the handling of agricultural products directly or indirectly untold millions of workers are involved These workers are liable to affection by the dust arising from hay straw grain tobacco peppers sugar cane chaff and cotton A group of diseases labelled asthma bronchitis and pneumonia may follow the inhalation of these dusts Despite the large amount of clinical material amongst people alleged to be suffering from farmer s lung thresher s lung grain fever tobacco dust disease paprika splitter s lung there is a paucity of well documented material Two conditions apparently related to the inhalation of sugar cane chaff and cotton have already been dignified with mouth filling names such as bagassosis and byssinosis

Bagasse Disease of the Lungs —Bagassosis refers to the respiratory disease which develops in dock workers or shredders of dried bagasse bales The respiratory illness is not found in workers handling the material after it has been heated The sugar cane from which the juice has been extracted is called bagasse Curiously enough the disease process apparently occurs only in workers handling bagasse derived from Louisiana sugar cane This vegetable fiber material is used in insulating board The illness which develops in these workers requires an exposure of several months to the dust has a sudden onset with sudden shortness of breath and cough productive of sputum sometimes blood tinged Shadows of reticulation or nodulation like those of miliary tuberculosis have been shown by *x* ray examination Radiological follow up on these patients indicate that the lung changes are reversible within two to four months

often matted together and produce wide bands of scar (Figs 67 and 68) Although doubly refractile particulate matter is scattered throughout the lungs, the site of this pigment deposition and particle size and shape are not of an incriminating nature

The secondary pathology associated with this unique fibrosis is the widespread appearance of bronchial hyperplasia and dilated alveoli filled with basophilic trapped mucus Fetalization of the epithelium overlying the parenchymal scar is a common finding In the regions of diffuse fibrosis, endarteritis obliterans is frequent Other nonspecific features found are confluent broncho pneumonia, and lipoid pneumonia Lipidization within the lungs is, probably, of an endogenic nature Only 1 fatal case out of 8 examined showed caseous tuberculosis No examples of lung cancer occurred Cor pulmonale is a frequent complication

Discussion —Due to the natural history of the disease and the unusual type of lung fibrosis it is accepted that this disorder is a dust caused disease The excessive diffuse fibrosis throughout the lung tissue favors a ' chemical dust' as the cause Although amorphous silica occurred in the furnace fumes, consideration of silica as the causative agent was abandoned for several reasons (1) The lungs in this condition lack the size, the nodulation the configuration and the stone like character of the silicotic lungs (2) Because of the integrity of the lymphoid collections within the lung it is postulated that in this condition the primary assault and brunt of the attack are borne by the septa, unlike the lymphatic involvement seen in silicosis (3) Although Costero has stressed the polymorphism of silicotic fibrosis in some fatal cases as due to organizing pneumonitis or transformation of atelectatic zones no pathological lesions resembling these are encountered *(4) Furthermore the dense strands of scar tissue can be traced back* to the matting together of thickened fibroblastic septa (5) Even in acute silicoses of the soapstone industry and in the Gauley Bridge disaster the lesions in the lung are reminiscent of silicosis (6) The anatomical pattern of this disease is diffuse whereas in silicosis of man and experimental animal the response is nodular *(7) No admixture of dusts such as carbon or iron with free silica ever completely* alters the nodular fibrosis producing a diffuse septal fibrosis

On the other hand there is some conflicting evidence Crombie has investigated the prophylactic inhalation of fine aluminum powder in mine workers over a period of three years and none of these workers developed pulmonary diseases I have performed autopsies on miners in Northern Ontario dead from natural causes who had been previously treated in the change houses with finely particulate aluminum powder and no specific lung changes were observed In the grinders of aluminum in aircraft manufacturing and in factory workers making aluminum powder in England Hunter and his associates have indicated that there was no obvious impairment of health The answer to these paradoxical observations may lie in the difference in particle size and concentration of dust in the working atmospheres

Animal Studies —Studies in the experimental animal have not resolved the confusion as to the basic action of amorphous aluminum Denny Robson and Irwin claim that the development of silicosis in the rabbit can be prevented with the introduction of finely divided aluminum into the quartz used for dusting On repetition of this work six years later Belt and King were unable to obtain such a protective action Irwin speculates that amorphous alumina has no pro

morphological grounds (5) Combinations of the foregoing factors the relationship between dust and an infectious agent other than as a mechanical carrier will require careful investigation and scientific probing

THE EFFECT OF SILICATES ON THE LUNG

The touchy problem of distinction between accumulation of dust phagocytes and the initiation of connective tissue proliferation reaches a histological zenith in the field of so called silicatoses The processing crushing bagging or sacking of these materials results in the formation of intense concentrations of dust in the atmosphere which are inhaled over a period of years by the laborers And yet, with long histories of exposures in such atmospheres the exact nature of the injuries produced in the lungs has not been established There are a number of reasons for this the radiographic evidence is equivocal post mortem reports are sparse and the experimental investigations have been limited

The most important of these silicates are Fuller s earth ($Al\,SiO_3$) China Clay ($H_4Al\,SiO_9$) Mica (aluminum silicates with potassium or magnesium) Sillimanite ($Al\,SiO$) diatomaceous earth and French chalk ($H_2Mg_3Si_4O_{12}$) or talc, including tremolite talc

Fuller's Earth —Several admirable detailed reports of the effects on lung following prolonged inhalation of this clay are in the recent literature The first post mortem report in medical annals is by Campbell and Gloyne, and quite recently a second case reported by Tonning Although the clay has a wide geographic distribution and is extensively used in industry (as early as 1892 Sherlock Holmes remarked in His Adventures that ' Fuller s Earth was a valuable product) the above reports are the only two contributions on the morbid anatomy

Fuller s earth is defined as any clay having a high capacity for removing color from animal vegetable and mineral oil expressed as (Mg Ca) O $Al_2O_3 5SiO_2$ (5-8) H_2O In the example reported by Campbell and Gloyne the constituent was a member of the montmorillonite group After this non plastic clay was mined the material was baked The mass exposure to this clay in the instance reported by Campbell and Gloyne was thirty eight years with a history of increasing dyspnea and cough for a year and half before death Gross examination revealed a number of dark colored nodules measuring $\frac{1}{4}$ to $\frac{3}{4}$ inch in diameter in all lobes of the lungs These soft nodules were poorly delimited crumbling in character and differed even to the naked eye from the lesion of silicosis Large numbers of dust particles were present in the bronchioles and air spaces These foreign body particles were ovoid and translucent ranging from 15 to 30 microns in diameter and doubly refractile with crossed Nicol prisms These particles were indistinguishable from the native Fuller s earth at the works A reticular proliferation surrounded the particles in the lymphatics of the lung (Fig 69) Moderate emphysema was noted No evidence of tuberculosis was encountered No appreciable trace of sericite or quartz was detected Middleton referred to these pathological findings as a lymphatic pneumonoconiosis without nodulation In Tonning s case in which the patient had not been exposed to Fuller s earth dust for fifty years dust reticulation foci were still plentiful after an exposure of fifteen years in his youth Native Fuller s earth is apparently not capable then of producing extensive collagenic fibrosis This lack of fibrosis accounts for the minimal x ray evidence found by Middleton and McNally and Trostler in

Because the disease is seldom fatal only rare opportunities have thus far been presented to determine the histological changes in the lungs

In humans serial biopsy studies and a single autopsy study have revealed localized pneumonic areas composed of macrophagic foam cells with foreign body reactions around the bagasse fibres

In experimental animals Gerstl Fager and Marinaro, through the inhalation route, produced extensive foci of necrosis and mononuclear cellular reactions *following the introduction of fresh bagasse material Bacteriological examination* of the fresh bagasse material and in the damaged lungs revealed numerous fungal mycelia and spores With autoclaved or formalinized bagasse only small foreign body granulomas were noted Hunter and Perry in a single autopsied case out of 39 exposed workers reported findings of bronchiolitis and bronchiectasis Histological examination showed ' nothing to suggest a disease of fungal origin No definitive report as to the specific incitant has been offered but future studies of biopsy and autopsy material should involve extensive cultural analyses and utilization of the periodic acid technic on fixed tissue to aid in the detection of fungal organisms or their shells

Cotton Disease of the Lungs —An acute respiratory illness of several weeks duration is acquired from handling cotton fiber in mattress manufacturing, cotton sacking and weaving concerns has been described in America by Neal and in England by Collis But a chronic form has been known for years, and is referred to by Lancashire cotton workers as Mondry fever or factory fever This chronic form is associated with long exposures in carding rooms of ten years or more increasing shortness of breath, productive cough and increased bronchovascular markings in the roentgenogram Dunn and Sheehan have published the only report on the pathological anatomy of this industrial disease Chronic bronchitis emphysema and cor pulmonale were the only findings These lesions were not in a way different from those observed in a chronic infective or allergic asthmatic An excellent review of this subject byssinosis has recently appeared in the British Medical Bulletin of 1950

Discussion — The cause in all these diseases is obscure A number of theories have been reported to explain this heterogeneous group of conditions (1) Allergenic because of the known frequency of vegetable dusts in inducing sensitivity and because of the nature of exposure to the dust of grains tobacco or cotton (2) Inhalation of different bacteria along with the vehicular dust Aerobacter cloacae has been found in the sputum of bagasse shredders (3) Inhalation of fungi Fawcett ascribes farmer's lung to a fungus Tornell claims clinical response of this pulmonary condition to the therapeutic use of the empirical fungicide potassium iodide Petri dishes exposed to the dusty atmosphere prevailing in cotton carding rooms grow a legion of fungi Jamison and Hopkins as well as Gerstl regard bagasse disease as a fungal condition Several instances have been seen of pneumonia following the cleaning of an old grain silo in which the workers breathed in a dusty atmosphere of pulverized grain Studies on these cases revealed a necrotizing pneumonia caused by Histoplasma capsulatum Finally there is no doubt that crops of grains rotting organic vegetable material or wet, then dried cotton and bagasse bales contain innumerable fungi and spores (4) Physical and chemical properties of the dust responsible for these lung conditions requires considerable investigation No diagnostic tissue findings have as yet been described to indicate that any of these conditions are separable on

morphological grounds (5) Combinations of the foregoing factors the relationship between dust and an infectious agent other than as a mechanical carrier will require careful investigation and scientific probing

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radiological surveys of workers at *Fuller's* earth plants The high base exchange mentioned by Policard, as an important property of Fuller's earth has had little influence on the septal connective tissue

China Clay —An important ingredient used in manufacturing of pottery, paint, soap and paper The bulk of China clay, a hydrated silicate of aluminum comes from Fowey, Cornwall and workers are exposed to the dust in shovelling the dried clay out of kilns As radiographic studies have not revealed any pro found mottling in these workers, and little disability has been credited to the dust, this dust probably evokes at the most, dust reticulation In King's experimental work with kaolin administered to small animals no fibrosis occurred

Sillimanite —Sillimanite, an aluminum silicate is obtained from crushed rock finely pulverized and calcined This material is used in the manufacture of porcelain Although Middleton, on radiological examination regarded the dust as harmless and produced only marked accumulation of dust phagocytes, two German workers, Jotten and Eickhoff, produced fibrotic nodulation in rabbits, which had been inhaling the experimental dust for 2 years



Fig 69 Fig 70

Fig 69 —Dust reticulation focus in China clay lung (× 132)

Fig 70 —Diffuse cellular and histiocytic proliferation following diatomaceous earth exposure (× 168)

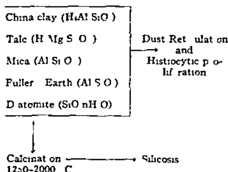
Diatomaceous Earth —Vigliani and Mottura in an extensive review of the role of diatomaceous earth in the production of a pneumonoconiosis add a single autopsy report Out of 7 individuals making candle filters all of whom showed radiographic shadows, there was 1 fatality The case was that of a male ex posed for eleven years to calcined diatomaceous earth dust and revealed pro nounced dust reticulation diffuse cellular and granulomatous proliferation in the septas perivascular and peribronchial regions (Fig 70) But above all there was hyaline sclerosis similar to that seen in massive quartz silicosis Al though the material employed contained 80 per cent diatomaceous earth the cause of the pneumonoconiosis lies in the micro crystalline silica content of the calcined diatomite X ray diffraction revealed the pattern of cristobalite The explanation for the hyaline fibrosis probably lies in the fact that ignition of native diatomaceous earth or Kieselgur converts amorphous silicon dioxide into micro crystalline cristobalite—a potent agent in the production of silicotic fibrosis both in the experimental animal and man

Nordmann reported 6 fatal cases of alleged diatomaceous earth pneumonoconiosis following inhalation although the crude diatomite was known to contain large amounts of quartz and asbestos[1] The lungs presented the pathologic findings attributable to quartz Legge and Rosencrantz noted radiographic shadows in 81 out of 118 workers in a diatomite quarry at Santa Barbara California where one of the largest deposits in the world exists The workers for the most part were Mexicans or Indians with a known high incidence of tuberculosis No autopsy studies were made More recent studies of this condition were presented in 1948 suggestive of a diffuse cellular proliferative lung disease

The experimental work up till Policard s researches in 1936 indicated that diatomite or tripoli produced nodular fibrosis Policard later supported by Duvoir and associates were unable to induce fibrosis in rats with pure diatomite dust In man and animal deliberate and accidental dusting with surgical talc initiates lamellar hyaline fibrosis

Tremolite Talc —In the cases reported by Porro Patton and Hobbs in 1942, of the 6 autopsies documented out of 15 examples of tremolite talc pneumonoconiosis there was in addition to diffuse reticular proliferation and numerous asbestos bodies (Fig 71) extensive scarring of a fibro hyaline nature with silicotic nodulation noted Of the 6 cases 4 showed active tuberculosis and case 5 reported as diffuse ' wheatena calcification with necrotic granulomas represents probably a fungal infection possibly due to Histoplasma capsulatum Again of the 4 out of 6 workers all had worked at least six years in iron lead and zinc mines previous to employment in tremolite talc mining From these facts it is difficult to label the alleged tissue effect due to talc, although the notation of diffuse cellular proliferation recalls the response seen by Vigliano in his post mortem report of diatomite pneumoconiosis The asbestos bodies owe their origin to the fibrous tremolite and anthophyllite components (asbestine) In the cases of radiological talc pneumoconiosis described by Eason and associates it is difficult to ascribe the changes simply to talc when the dust inhaled was a pyrophyllite containing 30 per cent quartz

Discussion —This histopathological silicate effect may be expressed in the following graphic fashion



To assess the role of silicates upon the lung a number of principles established or contentious must be considered (1) As Durkan has shown that calcination produces a change in the physical structure of diatomite and Gardner earlier had found that the *x* ray diffraction pattern of diatomaceous earth calcined at

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magnesium, and iron are represented in the relative proportions of approximately silica 40 per cent magnesium 40 per cent and iron oxide 3 per cent even within the lungs chemical estimations on cases of asbestotic fibrosis will be within this range

TABLE XI

	Amphibole		
	Blue Crocidolite South Africa	*Amosite*	*Chrysotile White Canadian*
Silica	50%	47%	41%
Alumina	1%	6%	3%
Ferric Oxide	35%	37%	2%
Magnesia	2%	6%	40%

In the processing of the mineral at the factories much of the iron is removed in the washing so that the fiber consists principally of hydrated magnesium silicate Any iron encrusted upon the asbestos fibers within the lungs is probably derived from hematogenous pigments liberated in the lung during the course of the disease

In the contaminated weaving sorting or carding rooms or even in the crushing houses the asbestos particles are inhaled in two forms Both varieties can be recognized in the lungs at histological examination the black granular pigment is that portion of the asbestos material still containing the unwashed iron moiety and forms a definite proportion of the inhaled particles

Etiology —The incarceration of the asbestos fiber in the bronchiolar and alveolar wall evokes an exudation of protein fluid and cells This outpouring of fluid is in response to the foreign body, perhaps to the silicates contained within the fiber The protein derived from the plasma and the iron from erythrocytic degradation contribute to the sarcophagus built around the fiber (Fig 73)



FIG 71 FIG 72

FIG 71 —Diffuse cellular proliferation and intra alveolar asbestos bodies in mixed tremolite talc mining (× 192)

FIG 72 —Diffuse pulmonary adenomatosis (alveolar cell carcinoma) and associated intraalveolar horse shoe shaped asbe tos body in an example of advanced asbestosis (× 19?)

Pathogenesis —The actual mechanism responsible for the production of fibrous tissue is not completely settled From the chemical aspect it has been suggested that hydrolysis by means of carbon dioxide and intracellular fluid leads to liberation of the magnesium moiety of the asbestos fiber in the form of a soluble bicarbo

1250° C showed the spectrum of cristobalite, it is difficult to exclude the possibility that cristobalite was not responsible for the pathological lesions observed (2) In the examples brought forward by Vigliano and other workers calcination of diatomaceous earth had occurred in the processing of the talc fillers (3) The occupational histories and the exact nature of the processing of the talc are not well chronicled (4) The well documented cases of prolonged inhalation of uncalcined Fuller's earth show only diffuse reticulation (5) The constant recurrence of the term "diffuse cellular proliferation" in all reports of talc or diatomaceous pneumonoconiosis suggests that this change along with dust reticulation represents the ultimate effects of native talcs (6) Other factors may of course account for the sweep of cellular proliferation, the existence of piezo electricity or rapid ionization of the talcs into more irritant forms of dust It is thus apparent that silicates particularly diatomaceous earth possess most probably the ability to produce a diffuse sweeping perilymphatic proliferative change but further investigations need to be done to substantiate such a conclusion

Asbestosis —*Historical Introduction* —Although Murray in 1900, Marchand in 1906 then Fahr in 1914 were the first to recognize that diffuse pulmonary fibrosis found at post mortem was associated with the inhalation of asbestos fiber it was not until ten years later that Cooke McDonald, Gloyne Wood Merewether and other investigators clearly established an etiological relationship between the mineral fiber and this crippling lung disorder To all intents and purposes asbestosis is a modern pneumonoconiosis modern industry has only been offering compensation within the last twenty years Murray's report attests to the extremely dusty atmosphere prevalent in carding rooms of absestos factories a patient suffering from disabling lung fibrosis on the wards of Charing Cross Hospital stated that he was the sole survivor of ten men who had started work with him in the carding room where the asbestos fiber was separated from other constituents of the crushed material

Clinical Findings —The clinical syndrome described by Merewether stressed the gradual onset of dyspnea dry cough and clubbing of the fingers The roentgenogram showed a shaggy outline of the cardiac shadow and in the lung fields a diffuse ground glass appearance Wyers has shown that with improvement of preventitive measures and plant hygiene clubbing of the fingers was greatly delayed but still found in 50 per cent of the afflicted individuals There was also an apparent increase in the development of lung cancer The x ray appearance has become more extensive and denser according to Linzach and Wedler Because of the lowered concentration of dust the disease requires greater periods of time for maturation as long as thirty five years in some individuals The morbidity rate has been markedly reduced through plant sanitation Although in Finland as recent as 1943 Wegelius demonstrated that 6 5 per cent of the workers in a carding factory suffered from asbestosis But in most countries working conditions have been vastly improved through rigidly enforced plant hygiene methods

Properties of the Mineral —Asbestos meaning unconsumable is a mineralogical vegetable known for thousands of years and utilized because of its insulating and fire resistant properties in manufacturing fire proof curtains shingles tiles and boarding A major source for this material has been the rich deposits in southern Quebec where there are intense concentrations of Canadian chrysolite The quartz rock is usually associated in the earth s crust with deposits of chrome iron and magnetite On chemical analysis the three elements of silica

from tissue oxidation and hydrolysis This outer crust is Prussian blue positive and responsible for the bizarre and irregular structures seen in the regions of densest fibrosis or within the alveoli Gardner and Cummings produced the asbestos body by treating the naked fiber with iron and silicate solutions "Fifty seven varieties' of shapes have been described by several authors particularly Glovne although the most frequent types encountered are discoid verruciform ' chicken bone ' ' bamboo bodies and ' gnarled or Indian club outlines Fissured segmented, crenated and moniliform bodies are also seen The golden yellow bodies may measure up to 300 microns in length and 5 to 10 microns in width with the central portion unstained and not reacting to Prussian blue in contrast the outer core possesses a marked affinity for histochemical stains specific for iron (Fig 73) These bodies are non refractile, so that polarizing light is not required for their demonstration

THE ASBESTOS BODY

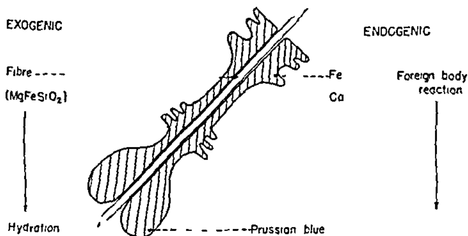


FIG 73

Animal Studies – The evidence that the diffuse fibrotic reaction in asbestos is due to the long fiber component in the amphibole and crysotile group of minerals is indirect The non fibrous moieties in these minerals such as magnesium calcium and iron are inert If a hydrated magnesium oxide known as brucite which exists in a long fibered form is experimentally introduced into small animals a pattern of fibrosis similar to that seen in asbestosis is seen Furthermore as silica is not present in brucite it cannot be incriminated as the fibrogenic agent Further support for the causation of the fibrosis as being due to the long fiber 200 micron is indicated by the fact that on crushing the asbestos fiber into fragments below 10 to 15 microns and then dusting under intense experimental conditions no fibrosis results This causative mechanism receives additional support in that other amphiboles or crysotiles although chemically different will produce asbestosis as long as they exist in the long fiber form

Sundius and Bygden point out that the long needles of titanium oxide which do not contain silicic acid cause formations identical to those seen in asbestosis

nate The iron is converted into an hydroxide and the essential silica is converted into the gel metasilicic acid As this gel possesses a high surface tension, it is formed at an irregular rate giving the material an irregular spheroidal and crenated appearance

On the other hand, most investigators favor a direct foreign body evocation of fibrosis Because of the large size of the structures they probably penetrate into the bronchioles and set up a foreign body reaction with ensuant fibrosis Certainly foreign body giant cells are frequent in this form of pneumoconiosis The lodgement of the asbestos fiber in these anatomical sites with the constant movement of the lung forces the asbestos particle in a needle like fashion into the bronchiolar and alveolar walls producing a direct traumatic effect, the alterations being the result of direct irritation and not due to an end effect through chemical solution of the silicate fiber

If the formation of silicic acid was responsible for pulmonary fibrosis, a pattern of silicotic nodulation would be expected

As the asbestos fiber is a large one, phagocytosis by giant cells is a prominent feature Distant transportation of the asbestos fiber does not take place, so that asbestos bodies are not observed in the fibrosed hilar lymph nodes

Pathological Anatomy —The lungs show a diffuse fibrosis particularly in the lower lobes, presenting a wet leathery appearance frequently nodular with a gray brown or black mottled appearance The location of the fibrosis in the lower lobes is explained on constant gravitational inhalation of the asbestos fiber The pleural cavities are frequently obliterated by dense tough adhesions with the pleura reaching up to 2 cm in thickness The diaphragm is involved also in this obliterating process Histopathologic examination in the earlier cases shows a peripheral subpleural and at times, a septal fibrosis, after several years the peri bronchial scarring and fusion fibrosis is the outstanding feature The peri bronchiolar fibrosis is most characteristic and is indicative of the principle site of action of the asbestos body The more recently reported post mortem studies of asbestosis emphasized that the fibrosis is not as advanced and symphysis of the interlobar fissures is not as pronounced as in fatal cases of twenty years ago Cor pulmonale is a frequent manifestation with extensive asbestosis

Direct secondary pathological alterations associated with this pneumono coniosis are of importance Within the lungs chronic bronchitis bronchiectasis bronchiectatic abscesses and pronounced emphysema are all frequently observed in addition of course to the obliterative changes of the pleural spaces Bacterial bronchopneumonia as an intercurrent infection is a frequent cause for the lethal termination of this condition Acute caseous tuberculosis is a frequent complica tion in this diffuse pulmonary fibrosis

Nature of the Asbestos Body —The highly characteristic structure within the lung is the translucent glistening fiber made up of a fibrous skeleton and the outer crystalline deposit This combined body forms the pathognomonic golden yellow structures known as asbestos bodies The naked fibers are the important ele ment from the standpoint of fibrogenesis Schuster has described asbestotic fibrosis in a dog which was used for ratting purposes in an asbestos carding plant the intense lung fibrosis in this dog was attributable to the naked crystals of the asbestos fiber Mavrogordato Gloyne and Gardner have shown by experimental studies that the outer crust is built around the inner core of the fiber This for mation is due to a colloidal interaction between fiber and body proteins derived

between the two conditions Contrary to this Perry believes that asbestosis favors the development of lung cancer His cases showed a frequent association between asbestosis and bronchogenic carcinoma of oat cell glandular or squamous cell pattern This relationship is not as readily accepted in America as it is in England But it would appear that there is a sequential developmental pattern between the two judging from individual case reports Such a post hoc, propter hoc argument warrants further search

Of itself asbestosis requires an intensive exposure to the long fibered asbestos dust over a period of approximately ten years to produce a crippling lung disorder Asbestosis alone is not usually accompanied by profound rapidly developing respiratory embarrassment If such a phase occurs in a known example of asbestotic fibrosis, there is usually a complication of infective or viral pneumonia tuberculosis or cor pulmonale from the vascular blockage with the onset of cardiac dyspnea

Prolonged cardiac failure according to Lendrum leads to focal siderosis with a granulomatous response These granulomatous foci made up of giant cells fragmented elastic tissue and encrusted with iron and calcium are frequently confused with asbestotic granulomas to the undisciplined eye These focal siderotic granulomas are usually at the site of the pulmonary bronchial arterial anastomoses which are functional in chronic pulmonary insufficiency These siderotic nodules in the lung parenchyma correspond to the Gamni Gandy nodules seen in the spleen in portal hypertension and are possibly of similar causation

As clumps of asbestosis bodies have been found in the sputum twenty three years after a one year exposure the diagnosis of this condition is mandatory and may be established by several means *e g* the needle puncture of the lung on those cases which show radiological evidence of widespread fibrosis, or similar acceptable signs Another maneuver worthy of trial is exfoliative cytological studies of the sputum with the demonstration of clumps of asbestos bodies intimately linked with elastic tissue

RECAPITULATION

From all of these canonical facts, it is possible to agree with Belt that the lung is a dossier filled with incriminating evidence In the following quotation although Belt is referring to the coal miner s lung the underlying principles are equally pertinent to the more recently recognized forms of pneumonoconioses

The collier s lung is in a very real sense his occupational log book it retains a qualitative and an indelible record of the mineral particles breathed during life and after death it constitutes a sort of palimpsest of the industrial history The collier s lung is like a parchment which has twice been written upon The original legend is in the form of dust reticulation and is inscribed over the whole fabric from top to bottom Though the penmanship varies it is set down always in the same spidery characters It is like the laborious uneven writing of a child now in a weak and faltering hand now with a bolder flourish The black dust which fills the role of a primitive sort of ink is heavily polluted with sandy grit The later legend is so to speak in a different tongue written still more unevenly in coarser characters and by a heavier hand These are the heiroglyphics of silica and tubercle Under this later influence the earlier record may well become smudged and distorted but it remains legible

As the asbestos needles of Finnish asbestos dusts are harder and more elastic, they produce a higher incidence and greater severity of lung fibrosis Despite the bulk of evidence supporting a mechanical causation for asbestosis as determined by Gardner and Vorwald's work, King and his associates reported that the intratracheal injection of long fibers (15 microns) into rabbits produces a nodular reticulinosis comparable with the silicotic nodule in experimental animals, and short fibers produced a diffuse interstitial increase in argyrophilic reticulin

Chemical Analysis —In an analytical assessment of the lung from a fatal case of asbestosis the contribution is limited In ashing the lungs, the chemical analysis reveals varying quantities of magnesium calcium iron oxides or aluminum Depending upon the partitional analysis it is possible to indicate that the contained minerals are compatible with an origin in, for instance the amphibole group, such as amosite crocidolite or a serpentine earth

Discussion —The development of asbetotic fibrosis occurs in the weaving carding and sorting rooms of the manufacturing plants Control of the dust in these sections of the process has markedly reduced the incidence of the disease Although the bulk of mining of asbestos occurs in Southern Quebec, no extensive series of asbestosis has been reported in the mining or rock crushing divisions of preparation

It is known that asbestos bodies are frequently expectorated in the sputum, without clinical or radiological evidence of the disease The mere presence of asbestos bodies in the sputum does not mean that a disabling lung condition is existent Just as a single swallow does not make a Summer an asbestos body in the sputum or an isolated silicotic nodule in a post mortem lung specimen does not constitute evidence of a crippling pneumonoconiosis

Tuberculosis principally of the acute caseous type is often associated with asbestosis and appears on morphological grounds to be an engrafted process upon previously prepared soil The presence of asbestos spicules within large caseous tubercles clearly indicates that the acid fast infection developed after the fibers had already been inhaled Tuberculosis from a review of the literature, appears to play an active role in approximately 30 per cent of the cases It can be seen then that tuberculosis although contributing to the lethal outcome in a fair proportion of examples does not afflict this fibrotic lung with the same intensity and incidence as is known for silicotic fibrosis In our series of silicotics studied, tuberculosis was found in 75 per cent of the individuals The lesions of tubercle are clear cut and there is no difficulty in recognizing the destructive damage wrought by the acid fast bacillus No alteration of the pre existent asbestotic fibrosis occurs with the complicating tuberculous infection The co existence of sarcoid has also been reported with a single instance of asbestosis but there is no definite causative linkage Pinkerton has reviewed a number of surgical specimens and autopsy material previously labelled sarcoidosis and has in a number of instances demonstrated Histoplasma capsulatum Thus fungal diseases should be considered in determining the nature of complicating necrotizing granulomatous inflammatory reactions seen in asbestosis

Recently a case of pulmonary adenomatosis was seen associated with numerous intraseptal asbestos bodies initiating a chain of thought on asbestos bodies cause and ultimate effect (Fig 72) Homburger analysed several of his own cases of asbestosis in which primary carcinoma of the lung was present and also considered previously reported examples concluding that there was no tell tale relationship

between the two conditions Contrary to this, Perry believes that asbestosis favors the development of lung cancer His cases showed a frequent association between asbestosis and bronchogenic carcinoma of oat cell glandular or squamous cell pattern This relationship is not as readily accepted in America as it is in England But it would appear that there is a sequential developmental pattern between the two judging from individual case reports Such a post hoc, propter hoc argument warrants further search

Of itself asbestosis requires an intensive exposure to the long fibered asbestos dust over a period of approximately ten years to produce a crippling lung dis order Asbestosis alone is not usually accompanied by profound rapidly develop ing respiratory embarrassment If such a phase occurs in a known example of asbestotic fibrosis there is usually a complication of infective or viral pneumonia tuberculosis, or cor pulmonale, from the vascular blockage with the onset of cardiac dyspnea

Prolonged cardiac failure according to Lendrum leads to focal siderosis with a granulomatous response These granulomatous foci made up of giant cells fragmented elastic tissue and encrusted with iron and calcium are frequently confused with asbestotic granulomas to the undisciplined eye These focal sider otic granulomas are usually at the site of the pulmonary bronchial arterial anasto moses which are functional in chronic pulmonary insufficiency These siderotic nodules in the lung parenchyma correspond to the Gamni Gandy nodules seen in the spleen in portal hypertension and are possibly of similar causation

As clumps of asbestosis bodies have been found in the sputum twenty three years after a one year exposure the diagnosis of this condition is mandatory and may be established by several means *e g*, the needle puncture of the lung on those cases which show radiological evidence of widespread fibrosis or similar acceptable signs Another maneuver worthy of trial is exfoliative cytological studies of the sputum with the demonstration of clumps of asbestos bodies inti mately linked with elastic tissue

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From observational and experimental facts and arm chair fancies written about carbon, silica and other minerals producing long disorders, an elemental skeleton has been created, which offers support for the more recent investigations of modern and distinctive pneumonoconioses (Fig 74)

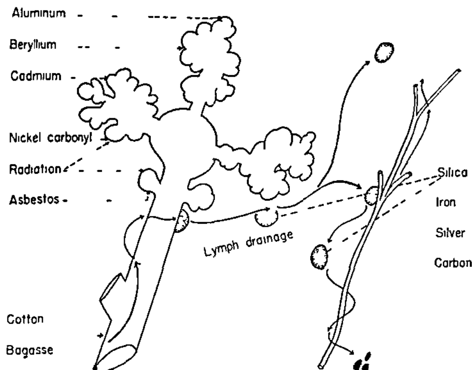


Fig 74

Occupational dusts of a non siliceous nature give rise to distinctive clinical and pathological disorders The starched trabeculae produced by Bauxite fume the mononuclear granulomatous response to beryllium or the perilymphatic cellular spread due to the fossilized diatom are highly characteristic patterns of disease encountered in this day and age The chemical pneumonias and the lung alterations related to moldy organic material are of a different nature These too await elucidation Of equal importance are the sequential complications of disabling lung diseases such as crippling heart disease or wasting lung cancer and these can be understood only if the background of the pathological changes is properly interpreted

It still remains paradoxical that the healing fibrosing response in the lung which brings succor and salvation to the consumptive brings disease and death to the good earth worker

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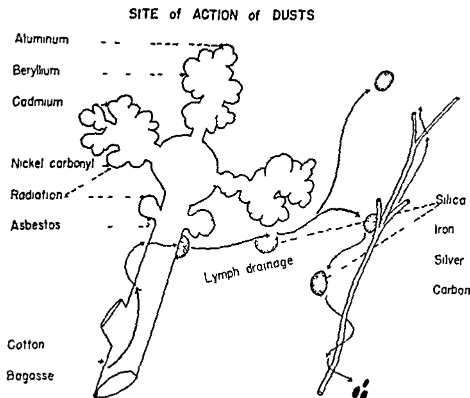


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